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MECHANISM OF LOCALIZATION OF VEGETATIONS OF BACTERIAL ENDOCARDITIS

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In studying vegetations in cases of acute and of subacute bacterial endocarditis [1] one is impressed by the constancy with which they are located on the valve surface facing the chamber which the valve serves, i. e., the outflow surface. The vast majority show predilection for the line of closure, but not infrequently the vegetations cover the greater part of the outflow surface. This has been appreciated for many years (Osler,[2] Clawson,[3] Clawson and others [4]). It seems, therefore, that nature must engineer such constancy by a definite mechanism rather than by chance distribution. However, from the diversity of the theories one may conclude that the principles of this mechanism have not been settled. The concepts that have been suggested may be outlined as follows: (1) coronary embolism, (2) mechanical forces such as eddy currents or impingement of valvular margins and (3) miscellaneous theories.

THEORIES

Coronary Embolism.—As far back as 1863 Luschka [5] described blood vessels in valves and correlated his findings of a richer vascularity in the mitral and aortic valves with the well established fact that endocarditis is most commonly found in these structures. This view was opposed at that time with the very modern objection that such valves were the seat of disease and thereby achieved their vascularity. However, in 1909 Coombs [6] presented reasons for believing that the organ-

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1. Allen, A. C.: Nature of the Vegetations of Bacterial Endocarditis, Arch. Path., to be published.
2. Osler, W.: Lancet **1**:415, 1885.
3. Clawson, B. J.: Arch. Int. Med. **33**:157, 1924.
4. Clawson, B. J.; Bell, E. T., and Hartzell, T. B.: Am. J. Path. **2**:193, 1926.
5. Luschka, cited by Kerr and Mettier.[8]
6. Coombs, C.: Lancet **1**:1377, 1909.

isms reach the valve through the coronary vessels. In 1917 Bayne-Jones [7] advanced evidence to show that valves are normally vascularized. This was confirmed by Kerr and Mettier [8] who injected india ink into the coronary circulation. They concluded that their studies lent "further confirmation to the embolic concept of endocarditis."

Recently Wearn and Moritz [9] published pertinent data from detailed studies of the vessels of valves. Of 235 hearts with no apparent inflammation, the mitral valve was vascularized in 50 per cent, the tricuspid valve in 31 per cent, the aortic valve in 5 per cent and the pulmonary valve in 4 per cent.

According to the embolic concept of endocarditis, one logically expects those valves to be most frequently involved which are most frequently vascularized. However, among apparently normal valves, the aortic was found vascularized far less often than any of the others and with about one-sixth the frequency of the tricuspid. And yet the tricuspid valve is the seat of endocarditis far less often than the aortic, even without antecedent rheumatic disease.

An additional objection—among others—to the coronary theory lies in the totally inadequate explanation for the localization of lesions on valves rather than on mural endocardium, not to mention the predilection for valves on the left side of the heart, and the constant selection of a particular part of the valve. The last part of this objection Eisenmann [10] meets with the statement that such highly selective localization depends on a peculiar distribution of the coronary vessels. Not only has this not been confirmed by subsequent elaborate studies but, as a matter of fact, there seems currently to be some doubt (Gross [11]) that normal valves are vascularized at all. It may then be concluded that this theory lacks adequate evidence as an explanation for the great majority of cases.

Eddy Currents.—Inasmuch as many of the lesions appear to begin near the line of closure, the explanation for the localization has been sought in eddy currents. But do eddy currents occur at this point? It is a fundamental physical principle that eddy currents tend to occur in a region of diminished pressure, particularly within a pocket. Adami and Nicholls [12] long ago pointed out that thrombi generally originate in venous valve pockets because of these currents. In the heart, pockets occur in two principal places (fig. 1): (1) between the superior

7. Bayne-Jones, S.: Am. J. Anat. **21**:449, 1917.

8. Kerr, W. J., and Mettier, S. R.: Am. Heart J. **1**:96, 1925.

9. Wearn, J. T., and Moritz, A. R.: Am. Heart J. **13**:11, 1937.

10. Eisenmann, cited by Hutyra, F., and Marek, J. F.: Special Pathology and Therapeutics of the Diseases of Domestic Animals, Chicago, Alexander Eger, 1912, vol. 1, p. 1080.

11. Gross, L.: Am. Heart J. **13**:275, 1937.

12. Adami, J. G., and Nicholls, A. G.: The Principles of Pathology, Philadelphia, Lea & Febiger, 1909, vol. 2, p. 68.

surface (or fibrosa) of the semilunar valves and the great vessels and (2) between the ventricular surfaces (or fibrosa) of the auriculoventricular valves and the wall of the ventricle. These are the sites of eddy currents (Best and Taylor[13]). However, both these areas are only exceptionally the seat of primary vegetations. It must therefore be concluded that eddy currents play no apparent significant role in the initial localization of bacterial vegetations.

Impingement.—According to this theory, the line of closure is frequently elected because of the trauma and strain due to mechanical impingement of the margins against each other as the leaflets or cusps slap shut (Leschke[14]; MacCallum[15]; Nedzel[16]). However, does significant impingement occur? To answer this question, it is important to review the anatomic background of bacterial endocarditis.

It is generally agreed (Libman[17]; Clawson[3]; Thayer[18]; Blumer[19] and others) that bacterial endocarditis occurs on a previously existing

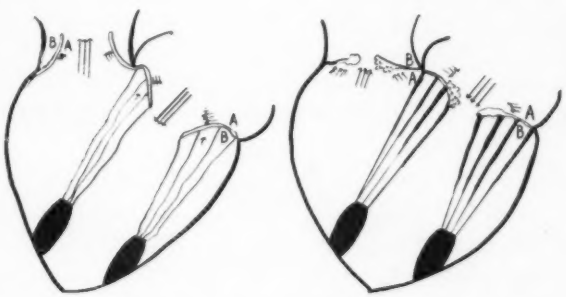

Fig. 1.—Diagrams illustrating the fact that very much more blood comes in contact with the outflow (*A*) than the the opposite (*B*) surface. It is on the outflow surface that vegetations localize with remarkable constancy. (Diagrams modified after Wiggers.)

valvular deformity in from 50 to 75 per cent of the cases. Such a deformity is usually a rheumatic fibroplastic distortion and occasionally a congenital anomaly. As is well known, the rheumatic deformity almost pathognomonically involves the free margins of the valves

13. Best, C. H., and Taylor, W. B.: Physiological Basis of Medical Practice, Baltimore, William Wood & Company, 1937, pp. 275-281, 183-187 and 454.

14. Leschke, E., in Kraus, F., and Brugsch, J.: Specielle Pathologie und Therapie, Berlin, Urban & Schwarzenberg, 1919, vol. 2, p. 1072.

15. MacCallum, W. G.: A Text-Book of Pathology, ed. 6, Philadelphia, W. B. Saunders Company, 1937, pp. 240-245.

16. Nedzel, A. J.: Arch. Path. **24**:143, 1937.

17. Libman, E.: J. A. M. A. **80**:813, 1923; M. Clin. North America **2**:117, 1918.

18. Thayer, W. S.: Johns Hopkins Hosp. Rep. **22**:1, 1926.

19. Blumer, G.: Medicine **2**:105, 1923.

(Antischkow [20]). A fibroplastic deformity of the free margin often produces stenosis and usually concomitant insufficiency. It is difficult to conceive, therefore, of much, if any, impingement occurring on the margins of a stenotic, insufficient valve during closure when these margins are unable even to meet (fig. 2).

This leaves at least 25 per cent of the cases of bacterial endocarditis to occur on apparently normal valves. But do even these normal valve leaflets impinge with the trauma and strain that is generally believed? The following is a description by Best and Taylor [13] of the mechanism of closure of the auriculoventricular valve:

> During auricular systole, the leaflets do not lie back against the ventricular wall but occupy a mid-position as a result of two opposing currents (the eddy currents reflected from the ventricular wall and the auricular stream). When, as a result of the fall in intra-auricular pressure, the incoming jet is diminished in force and finally ceases, the back eddy, persisting for a brief space and unopposed, ***approximates*** the valves or brings them together *gently*. They are not however firmly closed. This is effected by the rise in pressure in the ventricle when it contracts.

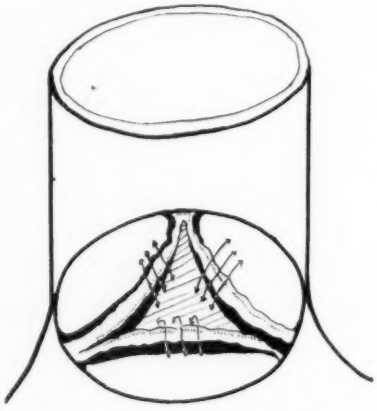

Fig. 2.—Diagram illustrating a stenotic, insufficient semilunar valve and the necessity for the line of closure to bear the brunt of increased impact since it is rigid and does not give way with the stream. Selectively increased contact and frictional resistance are also encountered in this area by virtue of the stenosis and insufficiency.

The trauma from closure after apposition has taken place is far less than that produced by edges which have been slapped together in the

20. Anitschkow, M.: Atherosclerosis (Lipoidosis) of the Heart Valve and Its Relation to Endocarditis, in Contributions to the Medical Sciences in Honor of Dr. Emanuel Libman, New York, International Press, 1932, vol. 1, p. 87.

manner of the general conception. Therefore, the impingement theory seems inapplicable not only to the lesions on the normal valve most frequently affected (mitral) but to the great majority of lesions superimposed on diseased valve leaflets, the margins of which are physically unable to impinge on each other.

Miscellaneous Theories.—Recently Nedzel [16] reported that he was able to produce endocardial lesions by the injection of pitressin with, and rarely without, a subsequent injection of bacteria. It is his belief that the pressor episode thus brought about causes the valve to exude from its surface a stringy adhesive substance to which bacteria adhere. The trauma produced by an augmented mechanical impingement is stated to be one of the chief factors in the production of the sticky streamers, inasmuch as during the "pressor episodes, the margins of the valve impinge on each other more forcibly" (Nedzel [16]). And yet, it is interesting to note that all of the lesions reported were on mitral valves, where the occurrence of significant trauma during closure is open to much question, as was indicated in the foregoing paragraph. Moreover, as mentioned, in stenotic, insufficient valves, such impingement cannot occur. Nevertheless, the whole problem of pressor episodes is intriguing, and the experimental data are valuable, although one may disagree with the interpretation thereof.

Grant, Wood and Jones [21] noted that degenerative changes tend to occur in congenitally bicuspid valves and in those with rheumatic deformities. These changes, they believe, lead to the occurrence of "platelet thrombi" which offer a foothold for bacterial implantation. This is a well recognized, keen observation. The question of whether these are truly platelet thrombi deposited on the valve or degenerative changes arising in toto from the valvular substance is not pertinent to the issue of present concern. The assumption that such projections may aid in localizing the bacteria is not unreasonable, although it does not account for the 25 or more per cent of cases of bacterial endocarditis in which the vegetations are generally assumed to occur on previously normal valves. Nor is it unreasonable to believe that the production of these changes is intimately related to the selectively greater "wear and tear" suffered by the valvular outflow surfaces where these "thrombi" were found. This will be discussed further in later paragraphs.

A variety of other possibilities have been suggested. For example, according to Babcock,[22] the rheumatic valve is less vascular, with the consequence that fewer phagocytes are able to reach and destroy the bacteria. Coombs,[6] on the other hand, believed these valves to be more

21. Grant, R. T.; Wood, J. E., and Jones, T. D.: Heart **14**:247, 1927.

22. Babcock, R. H.: J. Michigan M. Soc. **12**:645, 1913.

vascular with a resultant increase in opportunity for infection. Poynton [23] stated that the organisms lie latent in the rheumatic valve until caused to flare up with the consequent production of the characteristic bacterial endocarditis. Finally, the conception of allergy in the broadest sense of altered reactivity of tissue is gaining favor on the basis of convincing experimental evidence (Sémsroth and Koch [24]). Except the last, many such theories encounter distinct and obvious objections. However, even if these objections were met, none of the conceptions explains why the vegetations localize on one side of the deformity rather than on the other. In other words, the fibroplastic deformity, for example, in almost all instances is accessible on both sides at the distal, free margin. Similarly, the considerations of altered reaction of tissue, phagocytes and other such general factors apply more or less equally to the entire deformity. And yet, the surface facing the chamber served by the valve suffers implantation of bacteria with reliable constancy. Apparently, other factors are concerned.

In view of the multiplicity of theories, it seems desirable to set up the criterion that any theory attempting to elucidate the mechanism of localization must attempt to reconcile at least the following universally recognized facts relating to endocardial lesions:

1. In from 50 to 75 per cent of the cases, bacterial endocarditis is superimposed on a fibroplastic valvular deformity (Libman; Clawson; and others).

2. Congenital lesions are particularly susceptible to bacterial endocarditis (Abbott; [25] Osler [26]).

3. There is a distinct preponderance of lesions on the left side over those on the right side.

4. Bacterial endocarditis is rare in patients with auricular fibrillation secondary to rheumatic mitral stenosis (Libman; [17] Fishberg [27]).

No single theory has been offered thus far which explains all of these facts, and several of these facts are adequately explained by none of the theories. In the remainder of the paper, an attempt will be made to show that certain mechanical principles are concerned in each of the four groups of cases just cited and, of even more importance, that these principles are the same in all.

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23. Poynton, I. J.: Brit. M. J. **2**:306, 1920.

24. Semsroth, K., and Koch, R.: Arch. Path. **10**:867, 1930.

25. Abbott, M. E.: Ann. Clin. Med. **4**:189, 1925; Clinical Significance of a Congenitally Bicuspid Valve, in Contributions to the Medical Sciences in Honor of Dr. Emanuel Libman, New York, International Press, 1932, vol. 1, p. 1.

26. Osler, W.: Tr. A. Am. Physicians **1**:185, 1886.

27. Fishberg, A. M.: Heart Failure, Philadelphia, Lea & Febiger, 1937, pp. 338-346 and 441.

IMPACT AND CONTACT

For many years intracardiac tension has been thought to play a part in "reducing the resistance" of endocardial tissue, thus paving the way for implantation of bacteria. The occurrence of lesions in sites subjected to abnormally high tensions—for example, the right auricle with a patent foramen ovale or the right ventricle with a defective septum fibrosum—supports this general thesis. Gross,[28] who has treated the entire subject of endocarditis comprehensively, emphasized the factor of blood dynamics. The production of endocarditis by the injection of pitressin and bacteria (Nedzel) furnishes supporting experimental data. However, the term "hemodynamics" lends itself to facile usage. Precisely how do these dynamics bring about almost constant localization of vegetations at certain sites? The process, it is believed, entails more than simple tension. The attempt will therefore be made to analyze more exactly the nature of these dynamics, which may be resolved into two major components: (*a*) impact and (*b*) contact. Each of the four facts will now be examined separately to determine the role played by each of these components.

1. *Implantation of Vegetations on Rheumatic Endocardial Deformities.*—(*a*) Impact: In the normal valve, a good deal of the impact is warded off because the leaflets "give way" or play with the stream (fig. 1). In the rheumatic valve, however, the brunt of this impact is borne by the rigid, nonelastic parts, i. e., the line of closure, the usual site of the rheumatic fibroplastic deformity, because it is unable to "give" with the stream. The basis for this principle of mechanics is no less than Newton's third law of motion, which states that "to every action there is an equal and opposite reaction." In other words, the more fixed and unyielding an object is, the greater will be the impact produced against that object (Kimball [29]).

Therefore, as a corollary to this, any condition which appreciably alters the impact must be a factor, to the degree of such alteration, in enhancing the predisposition toward the localization of bacteria. Such an increase in the impact is produced in stenosis. For example, in aortic stenosis, "the velocity of ejection is greatly increased over normal —even during rest. The work of the left ventricle may be nearly doubled and the increase is due to the enormous increase in the kinetic factor" (Best and Taylor [13]). The impact is, of course, directly proportional to the kinetic factor inasmuch as both are a function of mass X velocity (Kimball [29]).

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28. Gross, L., and Fried, B. M.: Am. J. Path. **13**:769, 1937.

29. Kimball, A. L.: A College Text-Book of Physics, ed. 4, New York, Henry Holt & Company, Inc., 1932, pp. 19-24.

In addition, insufficiency is the usual concomitant of rheumatic stenosis. According to Fishberg, the diastolic backflow in insufficiency averages 36 per cent. According to Wiggers,[30] this backflow may vary from 5 per cent in small leaks to 50 per cent or more. This backflow increases the diastolic volume, and this, in accordance with Starling's law of the heart, causes a greater force of contraction and hence a correspondingly greater impact. In other words, in both stenosis and insufficiency—the common complications of rheumatic valvulitis—there is brought about an increased impact against the valve. The brunt of this impact is borne by the site of special interest—the fibroplastically deformed line of closure on which bacterial vegetations are commonly implanted.

(*b*) Contact: If, along with increased impact, a greater number of organisms forcibly brush against a certain part of the valve, the chances for bacterial implantation on that part naturally become greater. Therefore, the question to be answered is: Which part of the valve contacts the most organisms in a bacteremia?

In the first place, many more cubic centimeters of blood come in contact with the ventricular or outflow surface (spongiosa) of the normal or the deformed semilunar valve (and the outflow or auricular surface of the auriculoventricular valve) than with the opposite surface. This "opposite surface" (fibrosa) tends to be pressed against the aortic, pulmonary or ventricular wall, depending on the valve (fig. 1), and comes in contact with only a small fraction of the area of blood met by the outflow surface. (The pocket formed by the aortic wall and the valve fibrosa [fig. 1] is much too small to make eddy currents a significant factor.) By the same token, in bacteremia many more organisms will come in contact with this ventricular surface (fig. 1). As a matter of fact, vegetations that more or less completely cover this selectively contacted surface of a cusp without extension to the opposite surface are seen not infrequently, particularly in acute lesions. According to Swift,[31] the mitral lesions extend up over the wall of the left auricle in more than half the cases. These instances lend support to the concept that selective contact between valve and organisms occurs in this location and is important in the localization of vegetations. This same description of mechanism applies to each of the other valves.

Such is the situation when the valves are unthickened. However, a brief review of the mechanics discloses that in the stenosis and insufficiency produced by a rheumatic deformity there is brought about contact with an increased number of organisms at the line of closure.

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30. Wiggers, C. J.: J. A. M. A. **97**:1359, 1931.

31. Swift, H. F.: Endocarditis, in Nelson Loose-Leaf Living Medicine, New York, Thomas Nelson & Sons, 1932, vol. 4, p. 323.

In stenosis, three factors tend to work in this direction: (1) the diminished diameter of the stream as it passes through the narrowed orifice, (2) the increased stroke volume (Wiggers [32]) and (3) the lengthened systole (Wiggers [32]). The first two factors obviously increase the total surface area and hence the total number of organisms that rub against the projected portion or line of closure of the valve. The third factor increases the duration of such contact. All these elements by their very nature predispose toward bacterial implantation.

Over and above this, the insufficiency produces additional contact of organisms with the line of closure. This takes place during the diastolic backflow when, as the blood returns to its chambers, it again selectively brushes by the line of closure in vastly greater quantities than by the remainder of the valve (fig. 2). This diastolic backflow, as mentioned, may be considerable (from 5 to 50 per cent of the systolic discharge).

Furthermore, it is clear that any frictional resistance tends to make the contact between the valve surface and the blood stream more intimate. Such resistance is encountered in stenotic hearts. This is so because resistance is approximately proportional to the square of the velocity and inversely to the cross section of the orifice (Best and Taylor [13]). In rheumatic stenosis, there are present both an increased velocity and a narrowed orifice, each of which increases resistance, intimacy of contact and hence the chances for implantation of bacteria. The minute "platelet thrombi" described by Grant, Wood and Jones, may be an additional source of increased resistance.

So much for the majority of cases of bacterial endocarditis.

2. *Susceptibility of Congenital Lesions to Bacterial Endocarditis.*—The predisposition of congenitally abnormal valves to bacterial endocarditis is well known. However, in practically all such cases, with the exception of that of the bicuspid aortic valve, there is an obvious, self-explanatory defect in mechanics whereby abnormally great tension and impact are transmitted to a region not designed therefor, for example, a patent ductus arteriosus, a patent foramen ovale, septal defects and valvular stenosis. The predisposition of the "so-called congenitally bicuspid aortic valve," on the other hand, is not quite so easily explained. This is apparently not an uncommon finding. Thayer [18] reported an incidence of 7 per cent, and Lewis and Grant [33] one of 26 per cent, of bicuspid valves in their cases of subacute aortic bacterial endocarditis. Abbott [25] stated that of 40 hearts with bicuspid valves, 18, or 45 per cent, showed lesions of bacterial endocarditis.

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32. Wiggers, C. J.: Circulation in Health and Disease, Philadelphia, Lea & Febiger, 1937, pp. 686-690 and 652-653.

33. Lewis, T., and Grant, R. T.: Heart **10**:21, 1923.

Recently Gross [34] emphasized convincingly that these lesions, particularly those in adults, are most often on an inflammatory (rheumatic) basis—rather than congenital—with resultant fusion of cusps. This would tend, of course, toward stenosis and insufficiency. As a matter of fact, according to Abbott, stenosis and insufficiency *are* frequent concomitants of valves of this type. On this basis, the principles outlined in the foregoing discussion of the dynamics of stenosis and insufficiency are directly applicable to aortic bicuspid valves in adults.

3. *Predominance of Lesions on the Left Side.*—The predominance of lesions on the left side of the heart has long been appreciated. The difference in pressure on the two sides has been the most logical and most apparent cause given. However, if there were such a relationship here, one might expect the ratio between the incidence of right-sided lesions and the incidence of left-sided lesions to be approximately similar to the ratio between the pressure in the pulmonary artery and the pressure in the aorta. Therefore, the incidence of right-sided bacterial endocarditis was compiled from the combined series of Blumer,[19] Libman,[17] Clawson,[3] Horder [35] and Thayer.[18] In a total of 859 cases, 10.3 per cent involved the right side of the heart. The pulmonary arterial pressure is about one sixth or 16.6 per cent of that of the aorta (Wiggers,[32] Best and Taylor [13]). These figures—10.3 per cent and 16.6 per cent—are sufficiently close to be at least consistent with a possible relationship.

Another pertinent fact concerns the incidence of lesions on the right side in cases of acute and cases of subacute endocarditis. An analysis of Thayer's figures shows that there is a distinctly greater incidence of lesions on the right side in the cases of acute than in the cases of subacute endocarditis. Furthermore, Libman's [17] statistics reveal that 26.8 per cent of 56 cases of acute bacterial endocarditis involved the right side, whereas "only 1 in over 100" cases of the subacute variety were on this side. Kaufman [36] also stated that endocarditis of the acute type more commonly involves the right side. Although Clawson's [3] data do not confirm this conclusion, there is abundant evidence in support of it. In other words, there seems to be a generally greater tendency for the virulent rather than the relatively avirulent organisms (e. g., Streptococcus viridans) to involve the right side. The reason suggesting itself is that the *necessity for pressure, impact and contact diminishes as the virulence increases.* Additional evidence tending to substantiate this view may be had from Thayer's figures on preexisting valvular disease.

34. Gross, L.: Arch. Path. **23**:350, 1937.

35. Horder, T. J.: Quart. J. Med. **2**:289, 1909.

36. Kaufman, E.: Pathology for Students and Practitioners, translated by S. P. Reimann, Philadelphia, P. Blakiston's Son & Co., 1929, vol. 1, p. 30.

A preexisting deformity was found in 22 per cent of the cases of gonococcic endocarditis, 35 per cent of the cases of pneumococcic endocarditis and 45 per cent of the cases of staphylococcic endocarditis. On the other hand, such a valvular deformity was present in 82 per cent of the cases of subacute bacterial endocarditis due to streptococci. This also seems to indicate that the necessity for the deformity with the resultant changes in impact and contact diminishes as the virulence of the organism increases. That this should be so is perfectly natural and ties up with the entire concept herein advanced.

4. *Rarity of Bacterial Endocarditis in Patients with Auricular Fibrillation Secondary to Rheumatic Mitral Stenosis.*—Finally, there is the

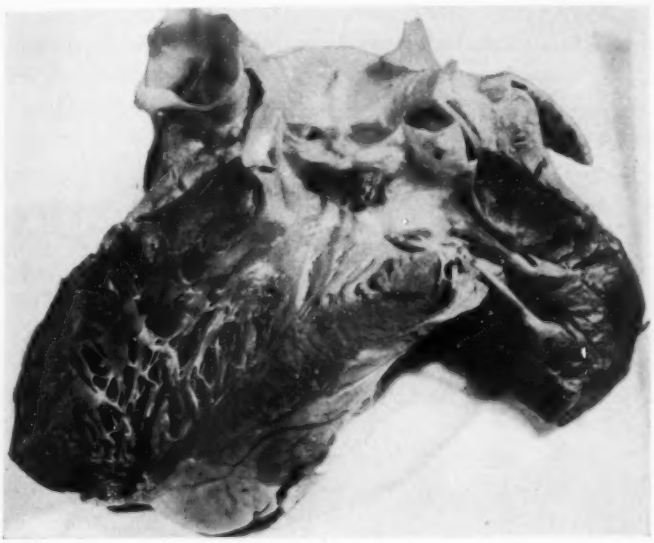

Fig. 3.—Acute bacterial endocarditis superimposed on a rheumatic fibroplastic deformity. This occurrence illustrates the importance of the mechanism of selective impact. Note that the vegetation localized beneath the projecting shelf, which bore the brunt of impact, rather than at the line of closure, which in this case happens to be thin, flexible and able to give way with the stream.

well recognized fact that bacterial endocarditis is rarely implanted in a fibrillating heart (Libman [17]; Fishberg [27]). Many equivocal explanations have been offered,[36a] among them the correlation with the degree of mitral stenosis, the integrity of the myocardium, the grade of immunity, the short life expectancy of patients with auricular fibrillation and mitral stenosis, and finally mere chance distribution. Each of these theories

36a. Segal, M. S.: Am. Heart J. **11**: 309, 1936.

has been challenged, and Fishberg concluded that no adequate explanation has thus far been offered. It may be of interest, therefore, to analyze briefly the circulatory dynamics of this arrhythmia.

In auricular fibrillation, "forcible ejection into the ventricle does not occur" (Best and Taylor [18]). By the very mechanism of irregular fibrillatory contraction, the important factor of resistance and impact against the valve is reduced to a minimum, as the blood drools through the valvular orifice. This of itself suggests a possible explanation for the failure of implantation on only the auriculoventricular valves. With regard to the semilunar valves, auricular fibrillation produces several changes in hemodynamics which reduce impact and contact with these valves:

1. Fishberg states that "the evidence is unequivocal that auricular fibrillation tends to decrease the cardiac output" (Smith and others [37]; Kerkhof [38]; Harrison and others [39]).

2. The ventricular rate is generally rapid, thus impeding diastolic filling and the force of contraction.

3. Finally, contractility is often not fully restored with each impulse; therefore the valve frequently opens feebly, as indicated by the pulse deficit.

In other terms, auricular fibrillation produces a distinct reduction in the potency of impact and frictional resistance, especially at the line of closure. It is maintained that the possibilities for implantation of bacterial vegetations are thereby correspondingly reduced. One does not wish to imply, however, that other factors may not play an auxiliary role.

COMMENT

In review, it is emphasized that any theory purporting to explain the mechanism of localization of bacterial vegetations must be challenged by at least four basic clinical and pathologic facts concerning endocarditis. Each of these has been discussed separately, and throughout the discussion the same principles have been employed to account for these facts in spite of their apparent diversity. However, it must be stressed that the concept of impact and contact attempts to explain not why implantation occurs but simply why implantation occurs at a particular site. It is felt that in all probability this localization takes place after the stage has been set by a generalized altered reaction of tissue or immunity. Evidence for this is furnished not only from clinical material

37. Smith, W. C.; Walker, G. L., and Alt, H. L.: Arch. Int. Med. **45**:706, 1930.

38. Kerkhof, A. C.: Am. Heart J. **11**:206, 1936.

39. Harrison, T. R.; Friedman, B.; Clark, G., and Resnik, H.: Arch. Int. Med. **54**:239, 1934.

(Swift[40]; Hector[41]) but from the sensitization experiments of Rosenow,[42] Semsroth and Koch,[24] Freifeld,[43] Birkhaug,[44] Kinsella,[45] Wright[46] and others.

SUMMARY

In from 50 to 75 per cent of cases bacterial endocarditis is superimposed on rheumatic endocarditis. Rheumatic endocarditis commonly produces a valvular fibroplastic deformity with stenosis. This lesion takes the form of a projecting shelf or barrier, usually at the line of closure, against which the blood stream strikes. By virtue of this obstruction to the systolic discharge (manifested by myocardial hypertrophy) the site of the deformity suffers a distinctly greater impact and contact than the normal valve leaflet, which "gives" or yields with the stream. This contact with the blood (and organisms in a bacteremia) is further enhanced by the diastolic backflow due to insufficiency—the usual concomitant of stenosis. The role of these dynamics in the localization of vegetations is suggested.

This same mechanism applies to congenital lesions including the "so-called congenitally bicuspid aortic valve."

Attention is called to the fact that the outflow surface of all valves, normal or deformed, comes in contact with a much greater area of blood (and toxic agents) than the opposite surface. The significance of this in the localization of vegetations is stressed.

An explanation based on the same principles is offered for the rarity with which auricular fibrillation is complicated by bacterial endocarditis. The possibility of the influence of other auxiliary factors is not precluded.

It is pointed out that there is an increased tendency for acute rather than subacute endocarditis to occur on (*a*) valves not previously deformed and (*b*) valves of the right side of the heart. This fact is correlated with the principles of impact and contact.

1 East One Hundredth Street.

40. Swift, H. F.: Am. Heart J. **3**:629, 1928.
41. Hector, F. J.: Arch. Dis. Childhood **1**:339, 1926.
42. Rosenow, E. C.: J. Infect. Dis. **7**:411, 1910.
43. Freifeld, H.: Klin. Wchnschr. **7**:1645, 1928.
44. Birkhaug, K. E.: J. Infect. Dis. **40**:549, 1927.
45. Kinsella, R. A., and Sherburne, E. C.: J. A. M. A. **80**:1643, 1923.
46. Wright, A. D.: J. Path. & Bact. **29**:5, 1926.

STUDIES OF HUMAN OVA

I. DESCRIPTION OF ARTIFICIALLY INDUCED PARTHENOGENETIC ACTIVITIES IN ONE

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The eggs of invertebrates are capable of parthenogenetic development, and many species normally multiply by this process. Not only this, but Loeb,[1a] Allen and Bonta [1b] and others have shown that the eggs of many species that do not normally multiply by parthenogenesis can be made to undergo parthenogenetic division by mechanical stimulation or by treatment with various organic acids and many other substances. For obvious reasons, mammalian parthenogenesis has not received as much attention. Loeb [2] and others have shown that parthenogenesis can take place in the ovarian eggs of the guinea pig, but there seems to have been no direct application of experimental methods to human tubal eggs for the purpose of inducing cleavage or other cellular activities. The human tubal ovum has remained uninvestigated except as to its morphologic character.

From the pathologic as well as many other points of view parthenogenesis in the human ovum is of great interest.

For many years, dermoid cysts, teratomas, certain hamartomas and other tumors have been said to have parthenogenetic origin from ova, and this origin would probably be accepted as possible by most observers if some one would only find that the human ovum can, in fact, become active parthenogenetically. In his monograph [3] Bosaeus reviewed the literature and findings, detailed experiments on parthenogenetically

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In this investigation we were assisted by a grant for research on growth from Mrs. William Nax and by special equipment from an anonymous donor.

1. (*a*) Loeb, J.: Univ. California Publ., Physiol. **1**:7, 1903. For discussion and literature see Morgan, T. H.: Experimental Embryology, New York, Columbia University Press, 1927, pp. 537-593. (*b*) Allen, E., and Bonta, A. M.: J. Morphol. **48**:123, 1929.

2. Loeb, J.: Anat. Rec. **51**:373, 1932.

3. Bosaeus, W.: Beiträge zur Kenntnis der Genese der Ovarialembryone: Experimentelle Untersuchungen über parthenogenetische Ovarialgravidität bei Amphibien, Uppsala, Almqvist & Wiksells, 1926.

developed amphibian ova and reached the deduction that the human ovum can probably become active parthenogenetically. This is now shown to be the case as described in subsequent paragraphs.

In addition, the doctrines of multiple and excessive potency are of great interest to oncologists.[4] The potency displayed by the ordinary nonfertilized human ovum is nil; it is now apparent that the actual potency is greater than the ordinary realization of that potency, just as in all other cells in which there still remain some powers of differentiation.

Details sufficiently accurate for these experiments of the ovulatory period in the human female are sketchily known. Allen, Pratt, Bland and Newell,[5] by a study of five human tubal ova recovered on the fourteenth, fifteenth and sixteenth days and a study of the appearance of the follicles from which these ova were discharged, set the period of ovulation as between the eleventh and the thirteenth day after the onset of the last menses. With a potentiometric device Burr and Musselman [6] recorded a sharp increase in potential in the human female on the fifteenth day before the onset of the next menses, presumably making the period of ovulation the thirteenth day after the onset of the previous menses.

The best chance of obtaining freshly discharged ova seems to be within this period, although one of our ova was obtained on the twenty-fourth day after the onset of the last menses.

To date (Aug. 15, 1938) five have been recovered. Numerous observations have been made, which will be published when additional specimens have been obtained to clarify certain points, such as density of capsule and accumulation of albumin around the ovum. Meanwhile, the behavior of the one ovum which became parthenogenetically active on "stimulation" will be recorded. None of the other four showed any spontaneous changes like it; nor can we find any mention of such changes in human ova by a review of the literature or by personal questions.

METHOD

Through the cooperation of the surgical staff, selected patients scheduled for hysterectomy were submitted to operation on the fifteenth or the sixteenth day after the onset of the last menses. In some cases the specimen consisted of

4. Reimann, S. P.: Biology of the Cancer Cell, in a Symposium on Cancer: Addresses Given at an Institute on Cancer Conducted by the Medical School of the University of Wisconsin, Madison, Wis., University of Wisconsin Press, 1938, pp. 114-134.

5. Allen, E.; Pratt, J. P.; Bland, L. J., and Newell, Q. U.: Human Tubal Ova; Related Early Corpora Lutea and Uterine Tubes, Publication 414, Carnegie Institution of Washington, 1930; Contrib. Embryol. **22**:45-76, 1930.

6. Burr, H. S., and Musselman, L. K.: Yale J. Biol. & Med. **9**:155, 1936.

the uterus, both tubes and both ovaries; in some cases only one tube was removed. Regardless of the kind of specimen, as soon as possible after it was removed, the tubes were cut from the uterus. A no. 20 Luer type needle was inserted into the fimbriated end and 10 cc. of Locke's solution was gently forced through. The washings were collected in watch crystals. Ova rapidly settle to the deepest portion. Photomicrographs of the ova were taken at any and every stage which seemed interesting by a Leica micro camera attachment. In some cases the ova were transferred to a hanging drop of human serum and then placed on the warm stage of a microdissecting apparatus. In other cases the original watch glass was used. But further details of this will be published with the observations on the respective ova, together with experiences in the use of rabbit and rat ova.

DESCRIPTION OF PARTICULAR OVUM

The specimen was obtained July 18, 1938, from a colored woman aged 35 years, whose periods averaged twenty-eight days. The flow lasted usually five days and was profuse. On the fifteenth day after the onset of her last menses, a specimen was removed at operation, consisting of two fallopian tubes, a fibroleiomatous uterus and one ovary. The ovary was soft and pink-gray, and had been partially resected at a previous operation. The other ovary was not removed.

The tubes were cut from the uterus and then washed with Locke's solution into watch crystals. The ovum was identified under low power and transferred to a hanging drop of human blood serum to which had been added a droplet of ethyl ester of acetic acid. The preparation was then placed in a warm, moist chamber.

The ovum on first appearance was rather large and quite uniform (fig. 1 *A*). On closer observation, it was found to be expanded against the internal surface of the zona pellucida. The zona pellucida was clear and uniform, appearing as a shell totally investing the contained vitellus. There was no perivitelline space since the ovum was everywhere in contact with the zona pellucida. At one point on the peripheral margin of the vitellus, directly beneath the zona pellucida, there was a marginal darkness which appeared to be a polar body. When the ovum was rolled over on the opposite side, this body became more evident. On focusing at different levels a darker central portion was seen. There were no vacuoles, and as far as observation could determine the ovum appeared fresh. The cytoplasm was not uniform in composition, and thus the ovum was slightly older than other specimens. (In younger specimens the cytoplasm is said to be more homogeneous.) Various-sized yolk granules were seen, and variations in the color of these granules were also observed.

The cytoplasm of the ovum was largely composed of small yolk granules. These granules were quite translucent to light and were tinged faintly yellow. The larger granules were more opaque to light and were more deeply colored. One large chromophoric body was

observed near the margin of the ovum. This body appeared to have radiating strands directed toward the zona pellucida and toward the central portion. The largest strand was directed toward the zona pellucida while the smaller strands were directed toward the central portion of the ovum. The dark yellow granules were but four in number and were evenly distributed throughout the ovum.

Within a half hour after being first seen, the ovum was transferred to a hanging drop of freshly drawn blood serum, to which had been

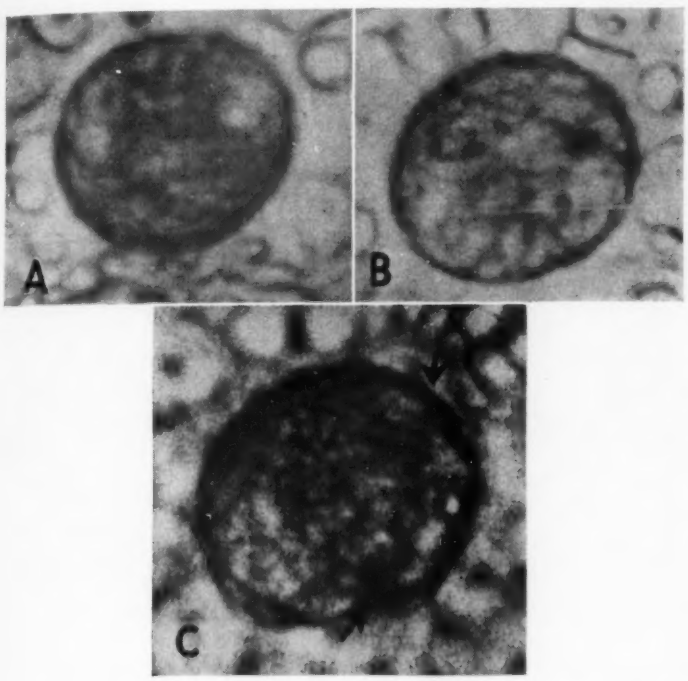

Fig. 1.—*A*, first appearance of the ovum, 89.6 microns in diameter; *B*, ovum rolled over on the other side to show chromophoric body; *C*, ovum rolled over with chromophoric body almost out of view to show furrow more distinctly; 4 mm. objective × 10 periplan. ocular; tube length, 160 mm.; photographic enlargement, × 11.

added a droplet of ethyl ester of acetic acid. This is known to stimulate parthenogenetic development in lower forms (Loeb). In transferring the ovum from Locke's solution a very small amount of calcium ion was necessarily introduced into the medium. The moist chamber was placed on the warm stage of the microdissecting apparatus, and both needles were brought into the field. The right hand needle was then brought to bear against the ovum and slight force exerted. The zona pellucida

offered some resistance to the entrance of the needle. As the needle was forced against the zona, a small indentation was produced, which disappeared just as soon as the needle was withdrawn. This seems to indicate that the zona pellucida is slightly elastic but tough. When more force was exerted on the vertical control, the needle entered the ovum very quickly. This jerky entrance into the ovum likewise indicates that the zona pellucida is tough.[7] On entrance the needle encountered no difficulty in further progress.

The needle was quickly removed from the ovum. Twenty minutes after the puncture the ovum underwent a definite cytoplasmic change. The zona pellucida became considerably thinner at one pole, while the

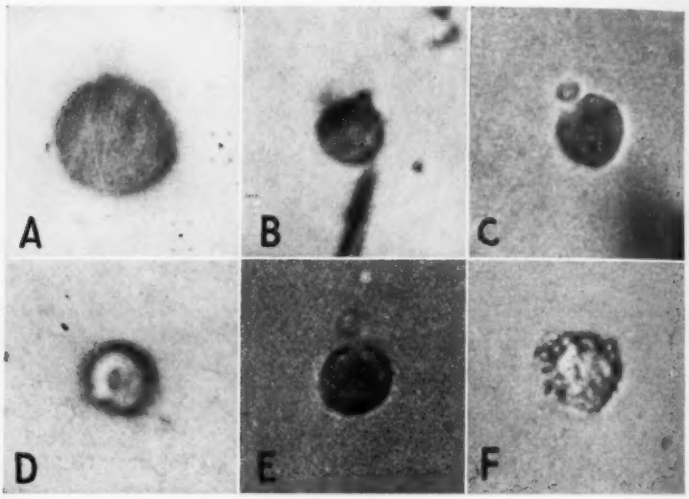

Fig. 2.—*A*, ovum when first transferred to microdissecting apparatus; *B*, ovum ten minutes after puncture; *C*, first extrusion, with crater in ovum; *D*, beginning of second extrusion; *E*, second extrusion; *F*, last stages in disintegration.

cytoplasm protruded directly beneath the now thinner zona pellucida. In about five minutes this cytoplasmic pseudopod had pushed itself out beyond the peripheral margin of the ovum and remained attached by a stalk, visible in figure 2 *B*. The base of the stalk then became constricted and in a very short time detached itself and assumed a spherical shape. Within a few minutes the small polar body migrated to a distance from the ovum (fig. 2 *C*).

The extrusion of the body left the ovum altered in appearance. That portion which had previously extruded the polar body now appeared as a

7. Dr. Georg S. de Renyi, in a personal communication, states that he has observed similar toughness in the rabbit's ovum.

very deep crater with a rough margin (fig. 2 *C*). Within five minutes the crater disappeared, and the ovum remolded itself. After another twenty minutes the ovum again showed signs of similar activity. A small peripheral granule again appeared directly beneath the zona pellucida (fig. 2 *D*). Although a bit slower than the first, the second body was also cast free from the ovum in about twenty-five minutes (fig. 2 *E*). After the extrusion of the second polar body, the ovum again showed a crater but one not quite as deep as the first one (fig. 2 *E*). The second polar body was a bit larger than the first but except for this difference was quite similar in appearance. After the extrusion of the second polar body, the ovum began showing changes that were undoubtedly stages in disintegration of the cell. Large peripheral vacuoles appeared in the region from which the polar bodies had been extruded, and the cytoplasm became increasingly more granular. The zona pellucida remained intact on the side that was not involved in the extrusion of the polar bodies. In figure 2 *F* the cell is shown about five hours old, in the final stages of disintegration.

During the course of this activity, another phenomenon was observed, which obviously could not be investigated in a stained preparation without sacrificing the other observations. A furrow was formed in the vitellus beginning from the dark chromophoric body previously mentioned (fig. 1 *C*). On one side the furrow was incomplete, but when the ovum was gently rolled over on its other side the furrow was seen as a moderately deep crater traversing the entire ovum (fig. 1 *D*). During the extrusion of the polar body the furrow increased in size but little. Its appearance was exactly that of the cleavage furrow seen in tissue culture cells. It is interpreted as the beginning of cleavage. More experience with other human ova, as well as with ova of other mammals, in relation to mediums, temperature and other factors have now made it possible to keep ova in better condition over greater lengths of time. Further experiments will probably lead to more complete cleavage, such as we have seen in a recent experiment in which we used the same method with a rabbit's ovum.

COMMENT

Polar body fragmentation of the type just described has been observed in the unfertilized ova of the mouse and rabbit.[8] According to Pincus, this type of activity represents a very irregular type of segmentation.[9] As far as we can discover, it has never been observed as a spontaneous process in the human tubal ovum, nor has this process ever before been artificially stimulated in any mammal's ovum.

8. Pincus, G. G.: Proc. Roy. Soc., London, s.B **107**:132, 1930.
9. Personal communication to the authors.

Further, according to Pincus, polar body extrusion of this type is characterized by the formation of a deep crater. This we have clearly observed in the extrusion of both polar bodies. Thus when the division of the ovum into polar body and oocyte is about to take place, the spindle is arranged so that one pole is incorporated in that part of the ovum that is destined to be the polar body and the other part of the spindle remains eccentrically placed in the ovum. When division finally takes place, the pole of the spindle remaining in the ovum is rapidly pulled down into the central portion, causing the peripheral part of the ovum that was involved in the extrusion to be pulled down with it. This forms the crater.[9] For this reason we feel certain that the parthenogenetic activity of this ovum was truly that in which polar bodies are extruded. On the other hand, conclusive demonstration of mitosis is best obtained from a specimen which has been fixed and stained, procedures which, as stated, could not be carried out without sacrificing further observation. But other ova will be prepared in this manner, and the results will be reported in a later paper. Further reasons are that the activity could not have been a surface precipitation reaction, because if it had been due, for example, to the presence of a small amount of calcium ion it would have taken place many times faster than it did.[10] Then, the second polar body was extruded without any mechanical stimulation. The ovum was initially stimulated, and this process followed minutes after.

SUMMARY AND CONCLUSIONS

Five unfertilized human tubal ova have been recovered from fallopian tubes of patients operated on for fibroleiomyoma and other noninflammatory pelvic conditions especially on the fifteenth and sixteenth days after the onset of the last menses.

Parthenogenetic activities resulting in extrusion of polar bodies and the formation of a deep cleft in the oocyte are described as they occurred in one particular unfertilized tubal ovum after mechanical stimulation in a medium of human blood serum containing a minute trace of the ethyl ester of acetic acid. The details of observations and experiments with four others will be published later, when a number of additional specimens have been studied to clarify certain special points.

It is concluded that the human ovum is capable of being artificially stimulated to parthenogenetic activity.

The implications of this finding are many and varied. There are mentioned but two: its relation to dermoids, teratomas, etc., and its bearing on concepts of potency.

10. Heilbrunn, L. V.: General Physiology, Philadelphia, W. B. Saunders Company, 1937, pp. 79, 81 and 542-546.

RARE LOCALIZATIONS OF HYDATID DISEASE IN TURKEY

REPORT OF A CASE OF PLURIVISCERAL ECHINOCOCCOSIS

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Echinococcosis is not a rare condition in Turkey. The parasite that is usually met with is Echinococcus hydatidosus. No infection with Echinococcus alveolaris has been observed in Turkey that I know of. Hydatid disease in this as in other countries is usually seen most frequently in the liver and in the lungs. Rare localizations have been the subject of several publications. Içgören,[1] in a case of hydatid cyst of the left lung, observed roentgenologically a cyst of the left tibia with a fistula which had been caused by trauma. Microscopic examination of the contents of the cyst in the tibia proved the cyst to be due to the echinococcus.

Schükrü-Aksel [2] at a postmortem examination observed primary hydatid cysts in the spleen, which weighed 10 Kg., and in the mesenterium. Neither the liver nor the lungs showed any involvement. In the discussion of this case Avni Aksel mentioned the observation of a young boy who showed generalized echinococcosis of the peritoneal cavity after an operation for appendicitis; about 40 hydatid cysts were removed. Exploration of the liver and the spleen of this patient showed multiple cysts in both organs. Lutfi Aksu mentioned a necropsy in which generalized peritoneal echinococcosis was observed.

F. Kâmil (cited by Çetingil [3]) and Çetingil [3] each published a case of echinococcosis involving the kidney. In the case observed by the latter, hooks and leukocytes were seen in microscopic examination of the urine and of the fluid obtained by puncture of the pleura. On the sixth day of the patient's stay at the clinic he vomited 200 cc. of pus containing leukocytes and hooks. Even then no evidence of a cyst could be seen in any part of the lungs. The cyst of the kidney had perforated through the diaphragm into the lung. The patient was sent to the

From the Laboratory for Pathological Anatomy, Gureba Hospital.

1. Içgören, K. N.: Türk tib cem. mec. **1**:517, 1935.
2. Schükrü-Aksel, I.: Türk tib cem. mec. **2**:38, 1936.
3. Çetingil, A. I.: Türk tib cem. mec. **2**:61, 1936.

urologic clinic for nephrectomy. Nissen [4] observed periodic appearances of hydatid cysts in the urine. The right kidney of his patient was removed. The same author,[5] while operating on a patient for periodic obstruction of the common bile duct and cholangitis, had to remove multiple cysts after choledochotomia. A big echinococcic cyst of the convex face of the liver had been originating them.

H. Hamdi and Tevfik Saglam reported a case of primary hydatid cyst of the heart, and Hazim Bumin,[6] a case of primary hydatid cyst of the left breast in a woman 25 years of age. In the Hamdi Museum of the Gureba hospital there is a hydatid cyst, 8 cm. in diameter, in a liver with atrophic cirrhosis.

It appears from this account that there may be primary or secondary involvement of organs other than the liver or the lungs. It is an accepted fact that the "hexacanthe embryo" has to pass the first filter (liver) and then the second filter (lungs) before it can penetrate into the great arterial circulation. According to Sabadini,[7] the most frequent localizations are in the liver (75 per cent) and the second most frequent are in the lungs (10 per cent). This leaves only 15 per cent of the cases in which other organs, such as those mentioned in the foregoing review, are involved.

REPORT OF CASE

A 53 year old miller, born in Priştine, Albania, had been admitted to the second medical clinic of the University of Istanbul, Nov. 4, 1937. An exploratory laparotomy disclosed peritoneal echinococcosis. Removal of the cysts proved to be impossible. It was decided, also, that certain tumoral elevations of the diaphragm seen roentgenologically were hydatid cysts. The patient died April 29, 1938.

Necropsy.—The body was that of a hairy man, 170 cm. in length and cachectic. The abdomen was extremely swollen. The umbilicus was obliterated by the scar of an operation—a scar 15 cm. long, beginning in the epigastric region. When the abdominal cavity was opened, implantation cysts were seen between the skin and the rectus muscles and between the muscles and the peritoneum (fig. 1 *S*). The peritoneum contained about 500 cc. of a clear yellowish fluid and was filled with multiple cysts, adhering partly to one another and partly to the abdominal wall. The adhesions were usually so firm that the cysts could be separated only by the knife. It was impossible to separate those of the abdominal wall from those of the liver. Still the bile ducts were absolutely free and the bowels colored. The intestinal loops were almost loaded with cysts, and the small pelvis was filled with them. Nowhere was there a sign of intestinal obstruction. Here and there thin walled cysts, pedunculated, were hanging free in the peritoneal cavity. Some of the cysts consisted simply of mother cysts; others contained daughter and granddaughter cysts. A great cystic tumor, almost

4. Nissen, R.: Türk tib cem. mec. **2**:464, 1936.
5. Nissen, R.: Türk tib cem. mec. **2**:507, 1936.
6. Bumin, Hazim: Türk tib cem. mec. **4**:262, 1938.
7. Sabadini, L.: Les kystes hydatiques de la rate, Paris, Masson & Cie, 1936.

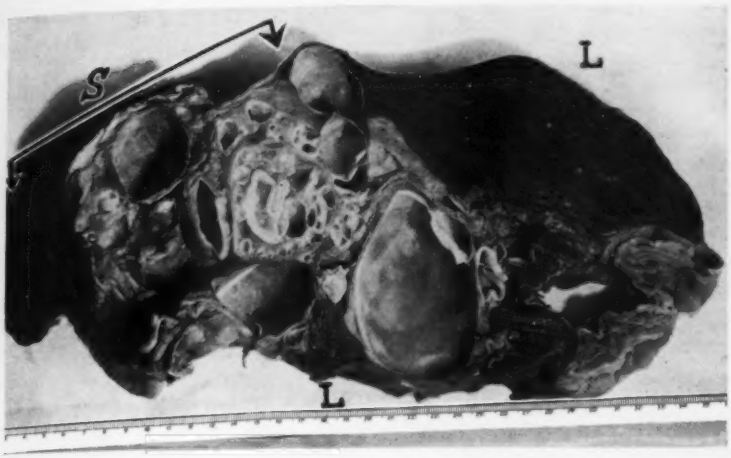

Fig. 1.—Hydatid cysts revealed in section of the liver, *L*. Note the bulk of cysts adherent to the lower surface of the liver and the abdominal wall, *S*.

Fig. 2.—Mass of peritoneal implantation cysts. *A* designates the appendix.

filling the left half of the abdominal cavity, proved to be the spleen. The diaphragm showed cystic involvement on both sides and was pushed up by the liver and spleen.

The liver with the cysts adhering to it weighed 5,220 Gm. The parenchyma of the liver contained several large cysts and was greatly reduced.

The spleen weighed 1,820 Gm. and contained equally numerous hydatid cysts, lined in part by the splenic tissue, which was increased.

The appendix had been obliterated by small cysts. The other abdominal organs were not involved.

The heart showed general hypertrophy and slight dilatation, especially of the conus arteriosus.

Both lungs adhered firmly to the parietal pleura. The right lung contained two sacciform bronchiectases toward its apex. In the upper lobe of the left lung a cavity almost the size of a Maltese orange was observed, which contained dirty greenish pus and drained into a bronchus. The pulmonary tissue surrounding it was infiltrated.

No localizations were found in the bones. There was general hydremia.

Microscopic Examination.—The clear colorless liquid of the cysts was centrifuged and found to contain rare hooks.

The cyst walls were constructed of rarely cellular, mostly collagenous connective tissue, showing inflammatory infiltrations of small lymphocytes and histiocytes. Inside of the outer cystic wall the inner cystic wall, consisting of concentric layers of chitin, formed many folds. No eosinophils were observed. It is interesting to note that their absence coincided with negativity of the Cazoni test; hence the patient must have been in a nonallergic state.

The wall of the abscess in the lung was of the same structure as the outer connective tissue walls of the cysts, so that it may be concluded that the abscess cavity was the remnant cavity of a hydatid cyst drained by the bronchus and suppurating secondarily. The content of the surrounding alveolar spaces was partly plasma and partly alveolar cells. The alveolar walls had been thickened by an increase in connective tissue. Some alveolar spaces had even been completely obliterated.

The liver cells had stored lipofuscin. Their protoplasm was granular. The spaces between them had enlarged. The reticular cells were edematous. In some places the chains formed by the liver cells were loosened, and single cells, isolated from one another, were to be seen. These alterations could be taken as preliminary signs of toxic hepatitis.

The spleen showed signs of fibrosis and stasis.

COMMENT

It is said that the frequency of hydatid cyst of the spleen shows geographic variations. Involvement of this organ is rarest in Iceland (according to Finsen, the incidence is 0.78 per cent) though in that country hydatid disease is of great frequency. Hydatid disease of the spleen is said to be more frequent in Russia than in Germany or France. The greatest incidence of the condition is said to be observed in Italy and Algiers, Algeria (4, 6 and 10 per cent, according to different authors). It is doubtful whether these figures have the significance of absolute values. In Turkey hydatid cyst of the spleen seems to be a relatively rare form of echinococcosis.

The first report of a case of hydatid disease of the spleen was published by Berthelot (1790). Sabadini [7] in his excellent monograph on this subject cited 288 cases that had occurred since that date. Among reports of more than 200 observations consulted, mention was made of only 10 instances in which the spleen presented a multiplicity of cysts. In 6 of these the spleen contained only two cysts; in the others, three, four and six. In the spleen described here twelve cysts of different sizes were counted, which shows it to be a rare specimen.

According to Dévé, a massive arrival of cysticerci in the splenic parenchyma is very rare. Sabadini proposed two pathogenic possibilities: (1) the passage of several cysticerci through the hepatopulmonary filters; (2) an exogenous vesiculation originating from the primordial vesicule. The first possibility has been accepted by many authors.

Splenic hydatid disease may be associated with other visceral localizations, as in the liver, the peritoneum or the liver and the peritoneum. The peritoneal involvement is secondary to perforation of hepatic or splenic cysts. Therefore the peritoneal cysts are called metastatic implantations.

The rarest type of plurivisceral localization seems to be simultaneous involvement of the lungs and spleen (Michon; Angelescu; Verequunov; Hermann—all cited by Sabadini [7]). Goyrand-d'Aix observed coexistence of splenic and pulmonary cysts with a cyst of the ligamentum latum uteri. Martin observed four cysts of the spleen, one of the lung and five of the omentum.

It is clear that the extent of the peritoneal generalization, the polycystic obliteration of the appendix, the multiple diaphragmatic, hepatic and splenic cysts coexisting with a drained but secondarily suppurating pulmonary cystic cavity make my case an instance of one of the rare types of plurivisceral localization. Concerning the splenic cysts, I believe that they resulted after the passage of cysticerci across the two filters. Concerning the peritoneal cysts, I think that they may have originated in two different ways: The first might have been by perforation from the liver and possibly also from the spleen, after which the cysticerci would have become implanted on the peritoneum next to these organs. As new vesicles were found hanging free in the peritoneal cavity, I believe that the aforementioned implantations gave rise, in turn, to exogenous growth of these vesicles.

SUMMARY

A short review has been made of cases of rare localizations of hydatid disease as recorded in Turkey. A rare case of plurivisceral echinococcosis involving the liver, diaphragm, left lung, spleen, peritoneum and appendix is reported and discussed.

EXPERIMENTAL ENDOCARDITIS IN DOGS

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Experimental endocarditis and its relation to the genesis of the disease in man has for more than fifty years been a problem not satisfactorily solved. The subject was brought to our attention when we encountered, quite accidentally, acute endocarditis in a dog following an intravenous injection of streptococci.

In experiments designed to produce meningitis, we were attempting to increase the virulence of a strain of beta hemolytic streptococcus by passing it through the blood stream of dogs. At autopsy of one of these animals we incidentally found vegetative endocarditis. Realizing that experimental endocarditis, despite much investigation, remains a matter of considerable confusion, we became interested in it.

Because much new work has been done in this field, it may be advisable to discuss briefly the present conception of the genesis of experimental endocarditis. According to the literature, positive results have been obtained by four general methods: (1) mechanical procedures with intravenous introduction of virulent bacteria, (2) simple intravenous injection of bacteria, (3) production of a bacterial focus in the body and (4) preliminary injection of predisposing substances, with subsequent intravenous injection of virulent organisms.

In the earliest instances experimental endocarditis was produced by mechanical means.[1] One method consisted in producing injuries of the valves with sharp instruments introduced through the great vessels and then injecting virulent organisms intravenously. Using this procedure, Kinsella and Hayes [2] produced endocarditis regularly. Another mechanical method was the intravenous injection of bacteria in mediums composed of large particles, such as pulverized carbon,[3] potatoes,[4] emulsion

From the Department of Pathology of the Nelson Morris Institute, Michael Reese Hospital.

1. Rosenbach, O.: Arch. f. exper. Path. u. Pharmakol. **9**:1, 1878.
2. Kinsella, R., and Hayes, C.: Proc. Soc. Exper. Biol. & Med. **24**:887, 1927.
3. Fulci, F.: Beitr. z. path. Anat. u. z. allg. Path. **44**:349, 1908.
4. Ribbert, cited by Lissauer, M.: Centralbl. f. allg. Path. u. path. Anat. **23**: 243, 1912.

of carcinomatous cells [5] or sterile flour.[6] Bonome [7] used cultures of staphylococci which grew in large clumps. He assumed that bacterial emboli, thus formed, caused the endocarditis.

The reliability of the simple intravenous injection of bacteria in the production of endocarditis varies in the hands of different investigators. Although sporadic positive results have been obtained, there is agreement among most workers in the field that this method is entirely undependable. On the other hand, Rosenow [8] reported endocardial lesions in 84 per cent of 44 rabbits receiving intravenous injections of large numbers of organisms isolated from patients with subacute bacterial endocarditis. As a matter of fact, most of the positive experimental results have been produced in rabbits; production of endocarditis in dogs by the simple intravenous method has been reported in very few instances. Lanfranchi [9] produced endocarditis in a dog by a single injection of staphylococci, and with two injections, administered four weeks apart, reproduced endocarditis in another dog. Fox [10] reported positive results in 3 dogs on injection of streptococci secured from patients with rheumatic fever and puerperal sepsis. Cornil, Mosinger and Haimovici [11] were able to produce endocarditis in dogs by a simple intravenous injection of streptococci isolated from patients with endocarditis, but they supplied no figures.

The experiments of Welch, Murdock and Ferguson [12] illustrate the third general method for the production of experimental endocarditis: They planted foci of Streptococcus viridans in teeth of rabbits, needled the hearts of the rabbits and sprayed the throats with influenza bacilli. Friedman, Katz and Howell,[13] using dogs, established a bacterial focus directly in the cardiac cavity by inserting through the thoracic wall a bakelite capsule containing blood agar cultures of Str. viridans. By this device they produced endocarditis. Birkhaug [14] reported the production of endocarditis in rabbits by introducing intramuscularly a focus

5. Panichi, L., and Guelfi, C.: Virchows Arch. f. path. Anat. **198**:449, 1909.

6. Viti, cited by Lissauer, M.: Centralbl. f. allg. Path. u. path. Anat. **23**: 243, 1912.

7. Bonome, A.: Arch. ital. de biol. **8**:10, 1887.

8. Rosenow, E. C.: J. A. M. A. **65**:1687, 1915.

9. Lanfranchi, cited by Rievel, H.: Ergebn. d. allg. Path. u. path. Anat. **17**: 2, 1915.

10. Fox, H.: Centralbl. f. allg. Path. u. path. Anat. **24**:529, 1913.

11. Cornil, L.; Mosinger, M., and Haimovici, H.: Compt. rend. Soc. de biol. **122**:685, 1936.

12. Welch, H.; Murdock, T., and Ferguson, J.: J. Lab. & Clin. Med. **21**: 1264, 1936.

13. Friedman, M.; Katz, L. N., and Howell, K.: Arch. Int. Med. **61**:95, 1938.

14. Birkhaug, K.: J. Infect. Dis. **40**:549, 1927.

of bacteria in blood agar and subsequently injecting the same organisms intravenously.

Recently much attention has been focused on the procedure which includes a preparatory injection of a substance thought to effect a predisposition to the development of endocarditis in the animal organism or in the valve tissues themselves. This preliminary injection of vaccine, casein or other substances usually administered intravenously, is followed after a varying period of time by an intravenous injection of virulent bacteria.

The efficacy of preparatory injections of specific vaccines has been investigated widely. The results have varied too much to be accepted without question. After establishing that intravenous injections of living cultures of streptococci always produced septicemia in normal rabbits, Cowan [15] did obtain endocarditis in 4 of 37 animals which had been previously immunized by nonlethal doses of living streptococci administered intravenously and subcutaneously. By this method, other investigators also occasionally obtained successful results. Dietrich,[16] for example, was able to produce endocardial lesions with staphylococci in 17 of 24 vaccine-treated dogs; Mair,[17] using pneumococci, observed positive results in 7 of 10 immunized rabbits. On the other hand, in two series of 31 immunized rabbits Wright,[18] working with living cultures of pneumococci, obtained endocarditis in only 1 rabbit.

Freifeld [19] demonstrated that the preliminary immunizing injections might also be nonspecific. Eight rabbits were prepared with streptococcus vaccine and then given injections of living cultures of Staphylococcus aureus. In 5 of these rabbits endocarditis developed. Silberberg [20] produced endocarditis in each of 5 animals, but Thomson [21] in only 2 of 12, both authors using colloidal dyes as preparatory substances and then injecting staphylococci. Other preparatory materials which have afforded some successful results are horse serum,[22] toxins,[23] epinephrine [24] and notably casein.[25]

Three noteworthy hypotheses have been offered in explanation of the effectiveness of the predisposing agent in the production of endocarditis.

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15. Cowan, M.: Brit. J. Exper. Path. **5**:226, 1924.
16. Dietrich, A.: Ztschr. f. d. ges. exper. Med. **50**:85, 1926.
17. Mair, W.: J. Path. & Bact. **26**:426, 1923.
18. Wright, H. D.: J. Path. & Bact. **29**:5, 1926.
19. Freifeld, H.: Klin. Wchnschr. **7**:1645, 1928.
20. Silberberg, M.: Virchows Arch. f. path. Anat. **267**:483, 1927.
21. Thomson, J. G.: Beitr. z. path. Anat. u. z. allg. Path. **95**:316, 1935.
22. Dietrich, W.: Virchows Arch. f. path. Anat. **299**:285, 1937.
23. de Vecchi, B.: Sperimentale, Arch. di biol. **86**:1, 1932.
24. Nedzel, A. J.: Arch. Path. **22**:724, 1936.
25. Semsroth, K., and Koch, R.: Arch. Path. **8**:921, 1929.

One holds that a certain degree of immunity must be produced in the experimental animal. Some authorities explain in this way the efficacy of preliminary treatment with specific vaccines. Wright [18] suggested that a local lesion on a valve can occur only in the presence of an immunity-inhibiting generalized infection of the body.

Another theory maintains that endocarditis results from the impairment of the reticuloendothelial function of the valvular tissue brought about by the vaccine, dyes, casein and other preparatory substances. In animals that had received intravenous injections of colloidal dyes,

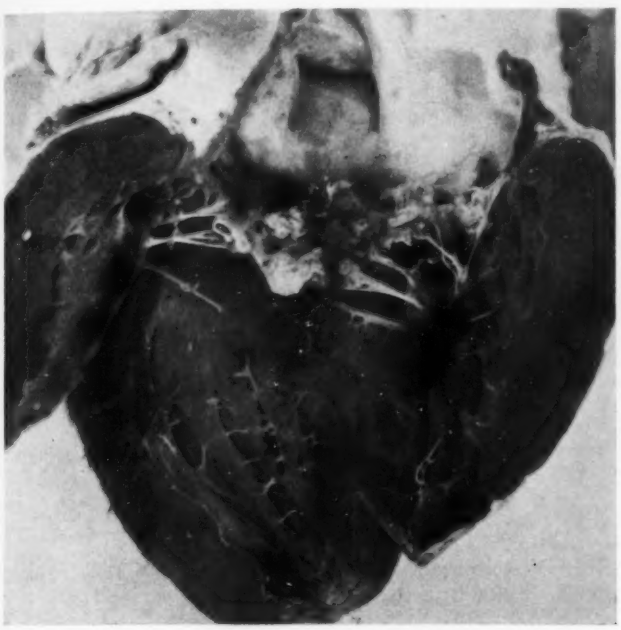

Fig. 1 (dog 20).—Acute endocarditis of the mitral valve. Note the large vegetations.

histologic examination of the cells in the valves revealed droplets of the dye. This is interesting in the light of Silberberg's [20] tissue culture experiments which showed that macrophages containing lithium carmine are impeded in their phagocytosis of organisms. Pfuhl [26] described pigmentation, vacuolation and destruction of histiocytes in the cardiac valves of animals treated with vaccines and casein. Semsroth and Koch [27] suggested that there may be a disturbance of the detoxifying ability of the valve tissue.

26. Pfuhl, W.: Ztschr. f. mikr.-anat. Forsch. **17**:1, 1929.
27. Semsroth, K., and Koch, R.: Arch. Path. **10**:869, 1930.

The third hypothesis is that the predisposition effected by the various preparatory substances consists in an activation (creation of a resorptive function) [16] or a sensitization [21] of the valve tissues and that the establishment of such a state is a prerequisite for the development of endocarditis. This explanation arose from histologic study of the valve tissues in animals given intravenous injections of the various preparatory substances only. The principal changes noted in these studies have been a definite increase in the size and number of the cells in the valves, appearance of histiocytes and edema of the valve tissue. On the other hand, Semsroth and Koch [27] were unable to confirm the reports of these histologic alterations of the valves in animals given injections of vaccine and casein.

From the foregoing review it can readily be seen that there is diversity in both the experimental and the theoretic approach to the

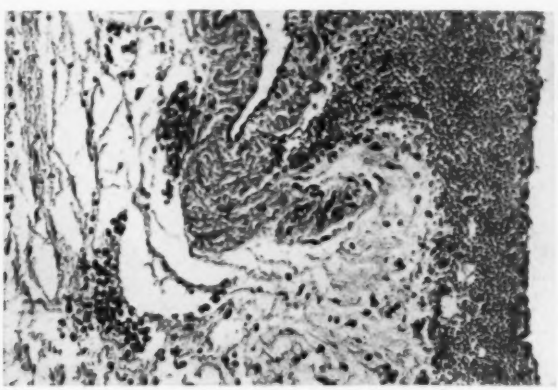

Fig. 2 (dog 5).—Section through the tricuspid valve. Note the hemorrhages and inflammatory cells. Hematoxylin-eosin preparations; × 110.

problem of experimental endocarditis, particularly in respect to the preparatory treatment. Because we found, quite by chance, endocarditis in a dog given an injection of streptococci obtained from another dog, we attempted to determine whether any preparatory treatment is, after all, an essential prerequisite to positive results; i. e., whether we could not achieve comparable results by the simple intravenous introduction or organisms a priori pathogenic for a dog.

METHODS

The organism we used was a beta hemolytic streptococcus isolated from a pneumonic lesion in a dog. A culture of streptococcus was sent to us by Dr. I. Pilot. The culture was in the smooth phase on blood agar. The organism fermented lactose and salicin but not mannitol or sorbitol. There was slight acid formation

in the presence of trehalose. The organism was a streptococcus of group C, according to the Lancefield precipitin test.[28]

Eighteen to twenty-four hour cultures of the streptococcus in beef heart dextrose broth were administered, the number of organisms per cubic centimeter of culture averaging between 1,500,000,000 and 2,000,000,000. The culture material was always thoroughly shaken to avoid embolic accidents. The bacteria were constantly passed through dogs. Occasionally we used twenty-four or forty-eight hour blood cultures (dextrose broth), positive for hemolytic streptococci, secured from other dogs given injections of our organisms.

The dogs were adult animals of the usual variety supplied to the laboratory. Injections were made into the superficial veins of the forelegs. Blood cultures were made when indicated.

After the death of an animal a postmortem examination was made as soon as possible. At this time cultures were taken from the heart blood in dextrose broth. Microscopic sections were made from the cardiac valves, myocardium and kidneys. Routine hematoxylin and eosin staining was done.

RESULTS

The accompanying table gives the results obtained in a series of 25 animals treated in the manner described. All of these subjects died spontaneously, apparently as the result of the injection of cultures of the streptococcus.

The table shows that unquestionable endocarditis occurred in 10 of the 25 dogs (40 per cent). In 8 of these, there was definite gross endocardial involvement. In 2 dogs only hemorrhagic foci were seen on the valve at autopsy, but subsequently histologic examination disclosed definite inflammatory changes. In 4 dogs endocarditis followed a single injection.

Of the other 15 dogs, 11 revealed bronchopneumonia or pyemic lesions, while the death of the remaining 4 dogs must be ascribed to bacteremia (or septicemia) alone. Blood cultures were positive for hemolytic streptococci during the course of injections as well as at autopsy.

The virulence of the bacteria varied during the fifteen month course of the experiments. The animals which were used in "stepping up" this virulence and which were therefore given injections of substandard organisms were not included in the series.

COMMENT

By simple intravenous injection of hemolytic streptococci cultured from a pneumonic lesion in a dog, endocarditis was produced in 40 per cent of 25 dogs. It is noteworthy that in 4 dogs endocarditis was produced after a single injection. The quantity of broth injected into

28. Topley, W., and Wilson, G.: The Principles of Bacteriology and Immunity, ed. 2, Baltimore, William Wood & Company, 1936.

Experimental Endocarditis in Dogs

Dog	Intravenous Injections	Amount of Each Injection, Cc.	Interval Between Last Injection and Death	Changes in Endocardium	Comment
1	4 in 4 days	12	2 days	No changes	Bacteremia
2	4 in 4 days	15	4 days	Acute verrucous endocarditis of mitral valve	Slight granularity; small foci of polymorphonuclear leukocytes with deposits of fibrin
3	1	25	2 days	Acute verrucous endocarditis of mitral valve	Slight granularity; polymorphonuclear leukocytes, scattered throughout valve; increase in mononuclear cells; edema; focal myocarditis
4	1	1.5	4 days	Acute ulcerative endocarditis of mitral valve	Acute myocarditis; multiple infarcts of spleen and kidneys
5	1	2	5 days	Acute endocarditis of tricuspid valve	Hemorrhagic discoloration; polymorphonuclear leukocytes, fibrin and edema
6	1	20	7 days	Acute endocarditis of tricuspid valve	Hemorrhagic discoloration; polymorphonuclear leukocytes, numerous mononuclear cells and fibrin; acute and subacute interstitial nephritis
7	3 in 4 days	14	10 days	No changes	Pyemia (abscess in muscles of chest)
8	4 in 5 days	16	1 day	No changes	Bacteremia
9	1	6 (sediment)	20 hours	No changes	Bacteremia
10	1	6 (sediment)	12 days	No changes	Bronchopneumonia
11	6 in 7 days	26	1 day	No changes	Pyemia (abscess in muscles of forelegs)
12	4 in 5 days	18	1 day	No changes	Bronchopneumonia
13	9 in 12 days	17	1 day	Acute ulcerative endocarditis	Lesion in wall of sinus of Valsalva
14	6 in 8 days	18	1 day	Acute thrombo-ulcerative endocarditis	Lesion in wall of sinus of Valsalva
15	3 in 3 days	11	1 day	No changes	Bronchopneumonia
16	3 in 4 days	6	2 days	No changes	Bronchopneumonia
17	4 in 4 days	12	3 days	Acute vegetative endocarditis of aortic valve	Myocarditis; infarcts of spleen and kidney
18	7 in 14 days	13	3 days	No changes	Pyemia (abscess in muscles of forelegs)
19	5 in 5 days	14	2 days	No changes	Bronchopneumonia
20	8 in 9 days	21	3 days	Acute vegetative endocarditis of mitral valve	Infarcts of spleen and kidneys
21	3 in 3 days	18	3 days	No changes	Bacteremia; acute focal glomerulonephritis
22	7 in 10 days	15	3 days	Acute vegetative endocarditis of tricuspid valve	Myocarditis
23	5 in 9 days	20	2 days	No changes	Bronchopneumonia
24	5 in 5 days	26	2 days	No changes	Bronchopneumonia
25	6 in 7 days	16	1 day	No changes	Pyemia (abscess in kidneys)

these 4 animals varied so greatly (from 1.5 to 25 cc.) that the number of the introduced streptococci could not have played an important role in the production of the endocarditis.

Heretofore this method has yielded sporadic and unreliable results at best except in a few isolated instances. Furthermore, although good results have been obtained in rabbits, only a few workers have reported positive results in dogs.

The question therefore arises as to why we were able to obtain such a large incidence of endocarditis in dogs with the simple intravenous injection. One possible explanation lies in the fact that we used streptococci isolated from another dog, while previous investigators worked with bacteria cultured from human sources. As stated, several workers have maintained that a certain degree of immunity in preventing extensive infection favors the localization of the inflammatory process on the valve. It is possible that in our experiments the dogs had some prior (natural or acquired) immunity to this organism, which, after all, was isolated from a dog. Such immunity, however, was obviously lacking in the dogs which showed no endocarditis.

Dietrich [16] suggested that endocarditis produced by the simple intravenous injection is due to "activation" of the valve by repeated previous injections of living organisms over a long period of time. This explanation is not substantiated by our studies in view of the production of endocarditis by a single injection in 4 dogs. However, in the other 6 dogs, succumbing within from six to twelve days after the first of multiple injections, the establishment of such activation might have been possible. It is also unlikely that a preliminary impairment of the reticuloendothelial function of the valve occurred, in view of the presence of endocarditis after a single injection. Furthermore our results cannot be explained according to the theory of elective localization, propounded by Rosenow,[8] inasmuch as the source of the organism was the lung of a dog and not the cardiac valve.

An important point to be considered is the application of these results to the development of bacterial endocarditis in man.

Undoubtedly, the mechanical treatment, particularly the technic of preliminary injury, gives reliable results in the experimental animal. However, this procedure is not analogous to the development of endocarditis on healthy endothelium-covered valves, as often occurs in man. Although the introduction of a focus of inflammation by the method of Friedman and his co-workers [13] also frequently led to endocarditis in animals, this procedure does not simulate the genesis of human endocarditis.

As stated, the hypothesis has been advanced in the last decade that there must exist a predisposition in the animal organism prior to the

development of experimental endocarditis. The actual production of endocarditis after preparatory treatment of the animal has been accomplished fairly constantly by some workers. Yet the data of the various investigators reveal that this method is not wholly dependable.

Our results, on the other hand, were secured without any preparatory injections. In fact, 4 of our animals acquired endocarditis after a single bacterial injection. The possibility of the existence of a natural predisposition to the development of endocarditis, of course, cannot be eliminated. Our work shows, however, that the artificial creation of a predisposing condition is not essential and that with organisms of sufficient virulence in the blood stream of animals of proper susceptibility endocarditis will develop even in the absence of any type of preliminary stimulating treatment.

The fact that bacterial endocarditis can be produced by so many methods with such a varied rationale suggests that a large number of factors may be involved in the development of this disease. Our experiments, however, clearly indicate that the presence of none of these several factors is essential and that virulent organisms per se in a previously healthy subject may be the sole cause of the development of acute bacterial endocarditis.

SUMMARY

Endocarditis was produced in 40 per cent of 25 dogs by simply injecting intravenously hemolytic streptococci isolated from a dog. In 4 dogs endocarditis was produced with a single injection. It is suggested that the use of a virulent strain isolated from the same species contributed to the positive results. It is concluded that numerous factors may be involved in the development of endocarditis. However, the presence of these several factors is not essential, for virulent organisms per se may cause acute bacterial endocarditis in a previously healthy animal.

PATHOLOGIC CHANGES IN RATS AND IN DOGS FED DIETS CONTAINING LEAD AND ARSENIC COMPOUNDS

COMPOUNDS USED: LEAD ARSENATE, ARSENIC TRIOXIDE, CALCIUM ARSENATE AND LEAD ACETATE

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This report contains the results obtained in an examination of four groups of rats and one group of dogs.

Each group of rats was comprised of four pairs. The members of each pair were litter mates, one of which was used as the experimental animal and the other as the control. The control in each pair was placed on diet A,[1] a diet relatively high in protein and composed primarily of natural food products, i. e., essentially of whole cereal grains, leguminous seeds and milk solids, so fortified in all accessory food factors that it met the requirements of the rat for growth and reproduction. The experimental member of each pair was also fed diet A, but with lead or arsenic added. The paired feeding method was followed.[2] The animals were placed on their respective diets at the age of 25 days. Since they were members of a larger group of animals, most of which were used for other investigations, the complete details of their history and treatment are presented elsewhere.

Group 1. The experimental rats in this group were placed on diet A, with the addition of 2.56 Gm. of lead as lead acetate per kilogram of diet, and were allowed to consume 500 Gm. of food, which required from six to seven weeks.

Group 2. The experimental animals of this group were placed on diet A, to which was added lead arsenate in such amounts that the lead content was 3.53 mg. per kilogram of diet. These rats were allowed to consume 1,000 Gm. of diet, which required from ten to twelve weeks.

From the Division of Pharmacology, Food and Drug Administration, United States Department of Agriculture.

Dr. G. E. Farrar was in charge of the pathologic investigations during the early stages of this study. Mr. C. E. Montgomery has been the technical assistant throughout the investigation. Dr. Esmond R. Long supplied the photomicrographs.

The investigation reported here was brought to a close long before it was complete by Congress when, on June 24, 1937, it passed the Agricultural Appropriation Act, containing the clause: "Provided further, that no part of the funds appropriated by this Act shall be used for laboratory investigations to determine the possibly harmful effect on human beings of spray insecticides on fruits and vegetables."

1. Laug, E. P., and Morris, H. J.: J. Pharmacol. & Exper. Therap., to be published.

2. Mitchell, H. H., and Beadles, J. R.: J. Nutrition **2**:225, 1930.

Group 3. The experimental diet in this group was diet A plus the equivalent of 215 mg. of arsenic per kilogram, added as the trioxide. The average time required to consume 500 Gm. was about six weeks.

Group 4. In this group the experimental diet was diet A with 215 mg. of arsenic per kilogram added as calcium arsenate. The time required by this group of animals to consume 500 Gm. of diet ranged from six to eight weeks.

Group 5. This group was composed of dogs. The report represents all the data we have had an opportunity to collect from a series of 29 dogs.

The history of the animals in the various groups and the data on their nutritional, chemical and therapeutic status will be published elsewhere: Group 1 and 2 (Laug and Morris [1]); groups 3 and 4 (Morris and Wallace [3]); group 5 (Calvery, Laug and Morris [4]).

Although this report is not as complete as we should like, several important observations have been made, some of which are confirmatory of data already reported in the literature, yet all of which may furnish information for others who are interested in the same problem. The fundamental histologic changes following continued ingestion of small quantities of lead and arsenic over long periods are almost entirely unknown. Where in this paper negative results are reported, it is well to keep in mind that more extensive studies or special technics might have revealed changes not demonstrated in this study.

GENERAL METHODS OF EXAMINATION

Except when otherwise stated, the blood was examined just before the termination of the experiment. Red and white cell counts were made by the usual technic, and hemoglobin was determined by the use of the Newcomber disk in a Klett colorimeter. Differential white cell counts were not made. Smears stained by Wright's or Giemsa's technic were carefully examined qualitatively for morphologic changes in the blood. In the blood of lead-treated animals and their controls a special search was made for basophilic stippling of red cells. Since in many of the experimental and control animals reticulocytes were relatively high,[5] comparative studies were not attempted.

The animals were weighed and then killed by decapitation. Qualitative observations were made of the carcass externally, and of the viscera, skeleton, brain and cord.

Blocks of the heart, lungs, liver, spleen, stomach, duodenum, ileum, large bowel, pancreas, testes or ovaries, kidneys and adrenals were hardened in Zenker's solution (without acetic acid) or in solution of formaldehyde U. S. P. diluted 1:10. The brain and cord were fixed in the latter solution. Embedding was done in paraffin, and sections for microscopic examination were stained with hematoxylin and eosin.

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3. Morris, H. J., and Wallace, E. W.: J. Pharmacol. & Exper. Therap., to be published.

4. Calvery, H. O.; Laug, E. P., and Morris, H. J.: J. Pharmacol. & Exper. Therap., to be published.

5. There is some evidence that at least a part of the rat colony was infected with Bartonella. Such an infection would explain the high reticulocyte count. Under normal conditions a high reticulocyte count is the only sign of a Bartonella infection.

The bone marrow was examined by the technic described by Farrar,[6] which consists in enumeration of the nucleated cells after quantitative dilution of the marrow with 1 per cent acetic acid. When properly carried out, this procedure seems to afford an accurate evaluation of the degree of marrow activity.

SPECIAL TECHNIC FOR EXAMINATION OF LEAD-TREATED ANIMALS

Preparations of the skeletal tissues were made according to a modification of the method reported by Sieber,[7] which consists essentially in the following: The bones are fixed from one to three weeks in solution of formaldehyde U. S. P. diluted 1:10 and saturated with hydrogen sulfide. When lead is present in large quantities, it becomes visible as black lead sulfide within a few minutes after treatment with formic acid. No lead sulfide is visible before addition of the acid. In a few cases, sections of the bone treated as described and impregnated with paraffin were prepared for microscopic examination.

Similar attempts were made to demonstrate lead in the soft tissues. With this end in view, blocks of the kidneys, spleen, liver, pancreas, intestine and brain were fixed for two weeks or longer in solution of formaldehyde U. S. P. (1:10) which had been saturated with hydrogen sulfide. They were then embedded in paraffin by the ordinary methods and cut into sections for microscopic examination. Where lead was present, it was seen as black deposits of lead sulfide, the visibility and localization of which were improved by counterstaining with 0.5 per cent saffranin in 50 per cent alcohol.

No special technics were applied to tissues of the arsenic-treated animals.

OBSERVATIONS

GROUP 1.—The experimental rats in this group consumed, over a period of from six to seven weeks, 500 Gm. of food containing 2.56 Gm. of lead as lead acetate per kilogram of diet. External examination revealed general impairment of nutrition and dulness of the hair. The mouths and teeth were clean and appeared normal, with no lead lines.

The hemoglobin was low (averaging 17 per cent less than that of the controls), and the red and white cell counts were slightly decreased (6.6 and 5.6 per cent, respectively). Morphologic examination of the blood revealed basophilic stippling of the red cells in specimens from all of the lead-treated rats, while this was not observed in the controls.

Autopsies revealed no gross changes in the viscera. There were, however, weight differences between some of the viscera of the experimental animals and those of the controls. The kidneys, spleens and hearts of the lead-treated animals as compared with the controls showed average weight increases of 22, 20 and 10 per cent, respectively, while the testes averaged 11 per cent less. These weight changes were consistent, being of about the same magnitude in each animal.

The kidneys were smooth and of normal color, the brains and cords appeared grossly normal, the bone marrow of the femurs was hyperplastic, being more red than that of the controls, and the skeletons

6. Farrar, G. E.: Am. J. Physiol. **117**:662, 1936.
7. Sieber, E.: Arch. f. exper. Path. u. Pharmakol. **181**:273, 1936.

in the fresh state revealed no abnormalities except that the bones of the experimental animals were very fragile. The intestines also appeared grossly normal except that a few centimeters above the ileocecal valve and extending throughout the cecum and large intestine the contents were black, due, undoubtedly, to the formation of lead sulfide.

A femur from each rat was preserved and decalcified in the presence of hydrogen sulfide as described in the section on pathologic methods. The results obtained were striking in that the entire femur turned black from conversion of the lead deposits into lead sulfide. One femur appeared spotted, black flakes appearing on the bony background, and under low magnification it was apparent that the black flakes represented deposits of lead sulfide about the haversian canals.

On longitudinal section of each completely darkened femur, the lead sulfide was seen to penetrate throughout the bony substance of the shaft, the marrow remaining free from discoloration. No lead line was present at the epiphysial junction.

One entire rat skeleton was treated according to the technic described. All the bones disclosed heavy deposits of lead sulfide, the heaviest being observed in the long bones, where deposits were more dense in the shafts than at the ends.

The cartilages of the thorax and those of the larynx and trachea also revealed heavy deposits of lead sulfide. However, smaller amounts occurred in the cartilages than in the bones.

Microscopic examination of the viscera gave negative results except as regards the kidneys. The changes in the kidneys were marked and were quite uniform in all specimens. The epithelial cells lining the tubules were very irregular in size. Some of the nuclei were tremendously hypertrophied, and in many of the nuclei eosin-staining bodies were present, similar to the nuclear inclusion bodies described by Blackman [8] in the kidneys of persons dying of lead poisoning. In some of the tubules these abnormal epithelial cells had sloughed into the tubular lumens. Occasionally several such sloughed cells had clumped together, forming a cast. The tubular changes were most marked in the medulla but were also present to a lesser extent in the cortex. They appeared essentially alike whether the material was fixed in solution of formaldehyde or in Zenker's solution. The glomeruli appeared uninjured, and the blood vessels were not sclerotic. No scars or inflammatory reactions were present.

Sections of kidney tissue which had been treated with hydrogen sulfide and counterstained with saffranin revealed particles of lead sulfide occupying some of the nuclei of the tubular epithelium. In a few cases, fine particles of lead sulfide occurred also in the cytoplasm. The lead

8. Blackman, S. S., Jr.: Bull. Johns Hopkins Hosp. **58**:384, 1936.

sulfide bodies within the nuclei resembled in size and position the eosinophilic inclusion bodies which were seen in sections stained with hematoxylin and eosin.

Throughout the medulla and to a lesser extent in the cortex there were large clumps of lead sulfide. Some of the clumps were round and somewhat larger in diameter than the renal tubule. Other clumps had an irregular shape. Some of the lead sulfide masses in the medulla were elongated and followed the directions of the renal tubules. It is probable that they represented casts of distended tubules around which the tubular structure had been destroyed.

Sections of the kidneys from the control animals were entirely free from all the tubular changes described for the experimental animals.

It is probable that the increase in weight of the kidneys of the lead-treated animals was caused by the modifications of the tubular epithelium.

Sections of the spleens which were examined by the prussian blue reaction revealed heavy deposits of iron in the lead-treated animals, while very little iron was demonstrable in the spleens of the controls.

Microscopic examination of cross sections and longitudinal sections of the femur revealed countless particles of lead sulfide. They were arranged, for the most part, in a radiating pattern about the haversian canals. Many particles occurred also in a zone beneath the periosteum, whereas none could be seen in the marrow. The black lead sulfide particles in the bone were easily distinguished under the high-dry and oil immersion objectives. Very small and sparsely distributed particles were most easily demonstrated by use of the dark field with an oil immersion objective. Sections of other organs and tissues revealed no definite changes.

GROUP 2.—The experimental rats in this group consumed, over a period of from ten to twelve weeks, 1,000 Gm. of food containing 3.53 mg. of lead as lead arsenate per kilogram of diet. All observations, both macroscopic and microscopic, were essentially normal.

GROUP 3.—Over a period of six weeks the experimental rats in this group consumed 500 Gm. of food containing 215 mg. of arsenic as the trioxide per kilogram of diet. The general nutritional state of all the animals was good. Autopsy revealed gross changes in the intestinal tracts of the arsenic-treated rats. While the small intestines of all the controls contained abundant food, those of the arsenic-treated animals contained only a colorless mucus. The cecums of all animals which had ingested arsenic trioxide were markedly distended and contained putty-colored mushy fecal material, while the large intestines appeared normal.

The nucleated cell counts of the bone marrow, averaging 2,556,000 for the experimental animals, compared with 2,284,000 for the controls, suggested slight hyperplasia of the bone marrow.[6] The skeleton

revealed no abnormalities, and the brain and cord appeared normal. Microscopic examination of the viscera revealed capillary dilatation in the lungs, kidneys, adrenals and intestines. The kidneys otherwise appeared normal.

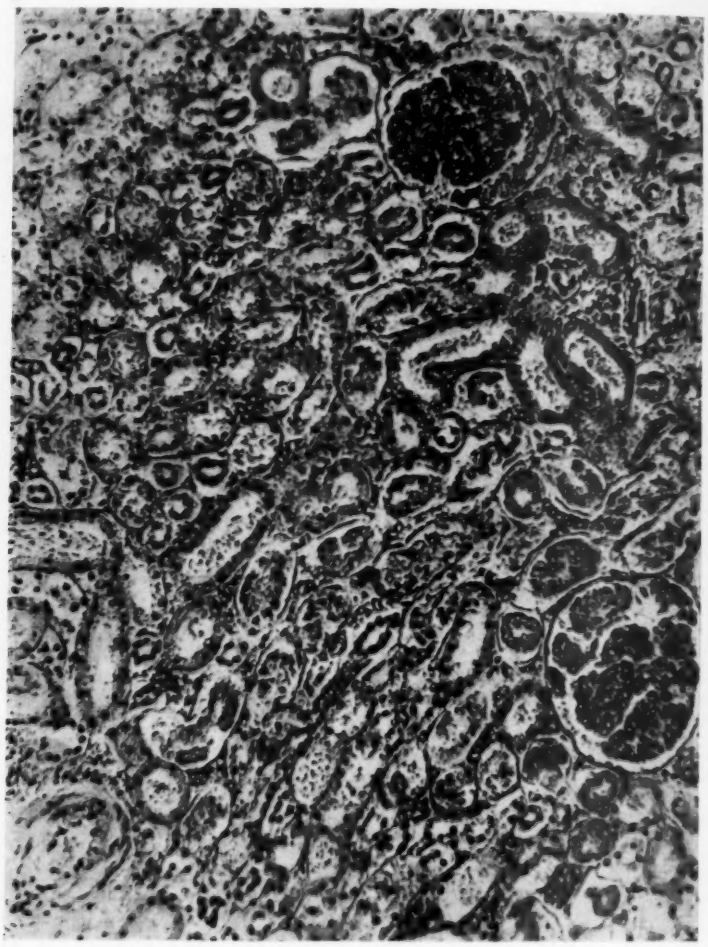

Fig. 1 (dog 1).—The photomicrograph shows protein in the tubular lumens and in Bowman's space in the glomeruli. In addition, tubular epithelium is found in Bowman's space. This is not entirely an artefact. It represents desquamation of the tubular epithelium at the outlet of the glomerular space, occurring as a result of injury. The subsequent forcing back into Bowman's capsular space is presumably an artefact induced in the postmortem examination.

GROUP 4.—The experimental rats in this group consumed 500 Gm. of food containing 215 mg. of arsenic as calcium arsenate per kilogram

of diet, over a period of from six to eight weeks. The animals receiving calcium arsenate were slightly larger than the controls. The teeth and mouths were in good condition, the paws and skin appeared normal, and the coats were glossy. The blood picture was essentially normal.

Postmortem examinations disclosed more fat in the abdominal walls and about the viscera in the treated animals than in the controls. There was edema of the abdominal subcutaneous tissue, peritoneum and intra-abdominal fatty tissue. The vessels supplying the intestines, uterus and testes were markedly dilated. The lungs were abnormally red from dilatation of the capillaries. The liver was enlarged, lustrous and grayish red, and had a watery appearance. The small intestines were extremely edematous, the edema being most pronounced near the ileo-cecal junction. The length of the small intestines was significantly increased in all of the arsenic-treated animals, and the cecum was markedly distended.

Nothing abnormal was seen in the stomach and large intestines. The mucosa of the small intestines was edematous, but there were no hemorrhages. The cecum in each of the poisoned rats contained large quantities of mushy yellow fecal material.

In the arsenic-treated females, the uterine horns were elongated, and the entire organ was flabby. No abnormalities were noted in the heart or pancreas. The spleen and kidneys revealed no change except that they weighed 18 per cent and 31.4 per cent, respectively, more in the arsenic-treated rats than in the controls. The brain, the spinal cord, the skeleton and the bone marrow of the femur appeared grossly normal in all cases. The edema in this group of animals was confirmed in the larger number used for chemical investigations,[3] and this accounts for the increased weight of the experimental animals as compared with the controls since the dry weights were the same.

Microscopic examinations were not made.

GROUP 5.—The histories of only a few dogs, which are representative of the whole group, will be given in detail. For a more complete presentation of the histories, symptoms, nutritional status and chemical findings, see Calvery, Laug and Morris.[4]

Dog 18.—This was a young animal, 46 days of age, when placed on diet B[4], a modified Cowgill diet, containing 64 mg. of lead as lead acetate per kilogram. The average lead intake per kilogram per day was 1.5 mg. On the forty-third day of the experiment, at 8 o'clock the dog showed the first severe signs of intoxication. When first observed, it began barking and failed to respond to the usual attempts to quiet it. When the cage was opened, it leaped out and ran wildly around the room, trying to find a place to hide. Shortly afterward mild attacks of convulsions occurred, which grew worse and occurred more frequently until the final severe attack at 1 p. m., when it died.

The autopsy was performed shortly after death. The carcass was that of a well nourished dog in opisthothonos with marked rigor mortis. There was foaming at the mouth. The skin was in good condition. The teeth were dirty, but the oral cavity appeared otherwise normal. The thoracic and abdominal viscera, including the kidneys, appeared grossly normal. The urinary bladder was empty. The brain was moderately edematous and hyperemic. Smears of the brain examined

Fig. 2 (dog 10).—The photomicrograph shows tubular degeneration with epithelial desquamation and marked proteinuria; × 240.

for Negri bodies revealed none. Postmortem blood smears stained by Wright's method revealed basophilic stippling in a few red cells. The skeleton in the fresh state appeared entirely normal. The marrow of the femur was abnormally red. Bones treated with hydrogen sulfide in solution of formaldehyde U. S. P. and decalcified in formic acid turned brown as a result of the formation of lead sulfide.

Microscopically, sections of the heart muscles appeared normal. In the lungs, liver and spleen moderate congestion was noted. The stomach, small intestine and colon disclosed nothing abnormal. No changes were seen in the pancreas or adrenal.

Sections of kidneys revealed nothing abnormal in the capsule, glomeruli or blood vessels. However, marked changes were present in the tubules. The

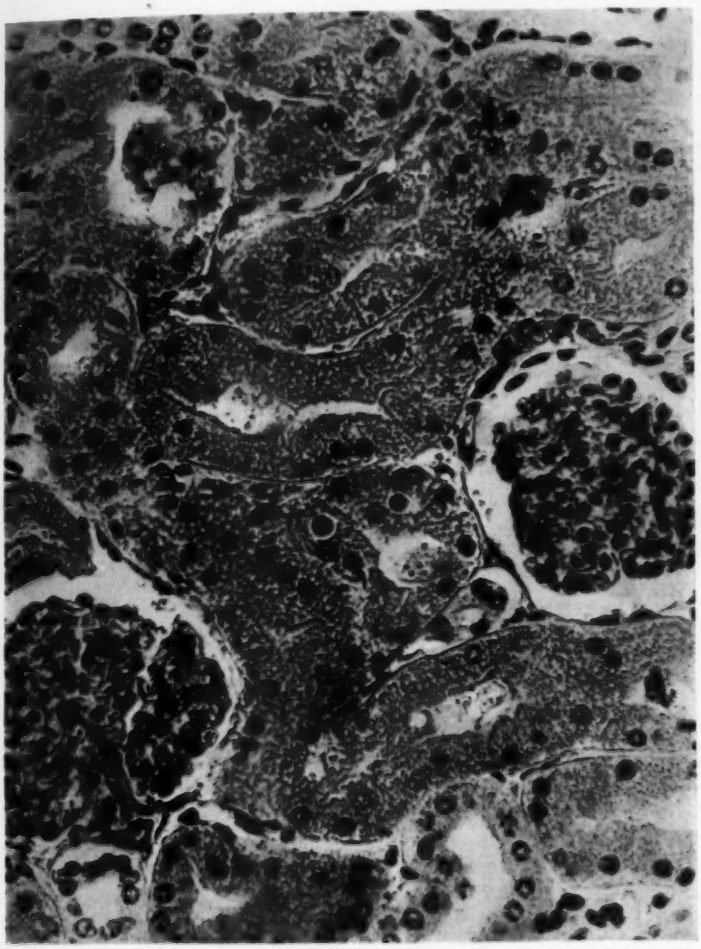

Fig. 3 (dog 18).—The photomicrograph shows mild tubular degeneration, with isolated cell injury; × 720. A striking feature is the presence of sharply defined spherical bodies, lying either in or adjacent to cellular cytoplasm, with about the size of the nucleus of a tubular epithelial cell. One such body is seen close to the center of the section (such bodies have been seen in human kidneys after long continued administration of lead and bismuth).

tubular epithelial cells were irregular in size, and many contained hypertrophied nuclei. Many injured epithelial cells had sloughed into the lumen. The changes noted in the epithelial nuclei were very different from those observed by us in

lead-poisoned rats and from those described by Blackman[8] in the kidneys of children who had died of lead poisoning, in both of which the characteristic change consisted in the presence of acidophilic inclusion bodies in the nuclei. No such bodies were noted in the kidneys of this dog. On the other hand, the damaged nuclei were broken into many fragments, which were scattered throughout the cell. The picture resembled what one would imagine to occur if an intranuclear explosion had taken place. A great many of the tubular epithelial cells were damaged in this way. In addition to the nuclear changes, inclusion bodies occurred in the cytoplasm. These closely resembled the bodies found by Pappenheimer and Maechling[9] after administration of bismuth. The picture was the same whether the material was fixed in solution of formaldehyde or in Zenker's solution.

Sections of kidney which had been treated with hydrogen sulfide while being fixed in solution of formaldehyde revealed small black particles in the capillary endothelium and in the basement membrane of the renal tubules. No lead sulfide particles were noted in the tubular epithelial cells. In this respect, again, the dog's kidney differed from that of the rats, in which lead sulfide particles were found within the tubular epithelial nuclei.

Sections of the brain and cord stained with hematoxylin and eosin revealed no cellular changes. The vessels appeared normal. Special staining for lead did not reveal its presence in the brain or cord.

A cross section through the shaft of the femur, which had been treated with hydrogen sulfide, showed particles of lead sulfide deposited around the haversian canals. A longitudinal section through the upper end of the femur revealed heavy deposits of lead at the junction of the diaphysis and epiphysis.

In a section of striated muscle taken from the quadriceps femoris there were small patches of lymphocytic infiltration between the muscle fibers. At the beginning of this experiment the red blood cell count was 6,375,000, and two weeks before death the count was 4,740,000.

Dog 8.—This dog was 123 days of age when placed on the same diet as dog 18. After thirty-one days on this diet, the animal went into severe convulsions, from which it recovered after being given 1 Gm. of magnesium sulfate intramuscularly followed by diet B with the addition of calcium. After the dog had been on this diet three days, it was placed on diet B for thirty-five days. After the total interim period of thirty-eight days, it was put back on the experimental diet. On the ninety-first day following the return to the experimental diet, it showed jerky muscular movements on the right side. It was also apathetic and passed urine which was bright red with blood. It was found dead in its cage the following morning.

The examinations of the blood of dog 8 are recorded in table 1.

The carcass was that of a well nourished, well developed dog. No rigor was present, and the carcass was no longer warm.

The thoracic and abdominal viscera appeared normal with the exception of the small intestines, spleen and kidneys. The intestinal mucosa was slightly hemorrhagic. The spleen was enlarged and firm. The kidney had a bluish gray appearance. The capsules appeared thickened but stripped easily, leaving smooth surfaces. The bladder contained bloody urine although the mucosa was intact.

The bones were hard and brittle. The shaft of the femur was markedly thickened, leaving only a small cavity for marrow. The marrow was small in

9. Pappenheimer, A. M., and Maechling, E. H.: Am. J. Path. **10**:577, 1934.

amount and grayish red. The skull was much thickened and coarsely porous. The large pores in the skull were filled with very red marrow.

The brain was extremely edematous, but the meninges appeared grossly normal.

Only the kidneys were examined microscopically, and these presented a picture like that described in the kidneys of dog 18; i. e., there was degeneration of the tubular epithelium with fragmentation of the epithelial nuclei.

Dog 7.—This dog was 141 days of age when started on diet B containing 12.8 mg. of lead as lead acetate per kilogram of diet and was on the diet for one hundred and forty days. The average intake of lead per kilogram of body weight per day was only 0.33 mg. The animal was found dead on the morning of April 1, 1937. There had been no signs of previous illness except that on the one hundred and nineteenth day it refused food for one day and on the day before death showed some apathy and no desire for food. The blood of this dog was not studied.

At autopsy the brain was hyperemic but not edematous. The teeth were dirty. The duodenal and jejunal mucosa was slightly hemorrhagic. There was bloody urine in the bladder although the mucosa was intact. The kidneys and the remaining viscera showed nothing grossly abnormal. The skeleton showed nothing of interest.

TABLE 1.—*Examinations of Blood of Dog 8*

Date	Hemoglobin, Gm. per 100 Cc.	Red Blood Cells per Cu. Mm.	White Blood Cells per Cu. Mm.	Results of Examination of Smear
12/12/36*	10	5,150,000	37,000	Many stippled cells; many nucleated cells, most of which showed basophilic stippling of the cytoplasm; moderate polychromasia; moderate anisocytosis
12/14/36	12.7	5,250,000	14,000	Many stippled red corpuscles; a few nucleated red cells; more polychromasia than in preceding smear
1/ 8/37	12.2	5,950,000	17,950	Many stippled red cells; no nucleated red cells; polychromasia as on Dec. 14.

* The dog had a convulsion on this date and was taken off the lead diet.

Only the kidneys were examined microscopically. They showed tubular degeneration similar to that described in the kidneys of dogs 8 and 18.

Dog 6.—This dog was 141 days of age when placed on the same level of lead intake (0.33 mg. per kilogram of body weight) as dog 7 and was found dead on the morning of April 27, 1937, after it had been on the diet one hundred and sixty-seven days. There had been no apparent illness except a refusal of food on the one hundred and fifty-third day and a show of some apathy as well on the day before death.

At autopsy the teeth were covered with a dirty brown deposit. There was a foul odor to the mouth.

The mucosa of the small intestine was hemorrhagic. The heart, lungs, liver and pancreas revealed nothing of interest. The spleen was slightly enlarged and firm. The kidneys were bluish gray. The capsule was slightly adherent, but the renal surface beneath the capsule was smooth. The bladder contained bloody urine but the mucosa was intact. The brain was hyperemic but not edematous.

The shaft of the femur was increased in thickness, and the bone was very hard. The marrow cavity of the femur was reduced. The marrow was small in amount and abnormally red. There was compensatory formation of marrow in the skull, similar to that described in dog 8 but somewhat less marked.

Microscopic examinations were made only of the kidney, and the observations were essentially the same as those on the kidneys of dogs 7, 8 and 18.

Dog 17.—This dog was a litter mate of dog 18 and was placed on the same diet at the same time. The first symptoms appeared at the fourteenth day as weakness of the front legs and mild paralysis of the right hindleg. The paralysis grew steadily worse until the thirtieth day, when both hindlegs were completely paralyzed, and the animal refused to eat. At that time it was taken off the diet containing lead and placed on a high calcium diet. The dog never recovered completely from the paralysis, although it greatly improved. After an interim period of two hundred and fourteen days, it was again placed on the lead-containing diet. During the second period of ingestion of lead the animal was bred and rapidly gained weight while showing the usual signs of pregnancy. At the end of the length of time of the usual gestation period, when the abdominal enlargement had disappeared and no pups were born, the dog was killed for further observation at autopsy. The blood picture is presented in table 2.

The carcass was that of a well nourished female dog. The skin was in good condition. There was atrophy of the thighs. The mouth was clean, and the teeth were all present, but some of the teeth contained small black blemishes which could not be removed by scraping.

TABLE 2.—*Examinations of Blood of Dog 17*

Date	Hemoglobin, Gm. per 100 Cc.	Red Blood Cells per Cu. Mm.	White Blood Cells per Cu. Mm.	Results of Examination of Smear
6/26/36	Not done	5,650,000	26,000	Negative
7/ 8/36	Not done	4,265,000	27,400	Negative
8/ 5/36	12.0	3,990,000	14,150	A few stippled red corpuscles (dog taken off the diet containing lead)
8/10/36	12.3	4,735,000	12,400	Many stippled red cells
1/ 8/37	13.2	6,250,000	11,300	No stippled red cells seen (dog returned to diet containing lead
4/29/37	14.6	6,700,000	11,500	Moderate number of nucleated red cells; moderate number of stippled red cells

Gross examination of the thoracic and abdominal viscera exhibited nothing noteworthy with the exception of the uterus. *Both horns of the uterus contained the remnants of resorbing fetuses.* The brain and the cord appeared grossly normal. The skeleton in the fresh state revealed nothing of interest.

Microscopic examination of the kidney revealed only moderate tubular changes.

Controls.—The autopsies on the control animals all disclosed essentially normal conditions.

COMMENT

Pathologic examinations have been made of groups of rats receiving diets containing different compounds of lead and arsenic. The animals in each case were taken from larger groups, the other members of which were used for chemical investigations, which are reported separately.[4]

The rats of group 1, receiving a diet containing 2.65 mg. of lead per gram until they had consumed 500 Gm. of diet, showed dulness of hair, moderate secondary anemia associated with basophilic stippling of the red cells, large deposits of iron in the spleen and a hyperplastic bone marrow. By a special method of treatment enormous quantities of lead were found in the bones and cartilages; large quantities were

also present in the kidneys, but none was demonstrated in other organs. The long bones were increased in thickness and were abnormally hard and brittle. This observation was extensively confirmed in the animals used for chemical investigations.[1]

The kidneys showed marked irregularity of the tubular epithelium, with hypertrophy of the nuclei of many of the tubular epithelial cells. Many of these nuclei contained eosinophilic inclusion bodies similar to those described by Blackman [8] in the kidneys in cases of lead poisoning in man. Inclusion bodies also occurred in the cytoplasm. These closely resembled the bodies found by Pappenheimer and Maechling [9] after administration of bismuth. Special treatment of the kidneys with hydrogen sulfide revealed lead inclusion bodies in the nuclei, which were similar in size and position to the acidophilic bodies mentioned. It is believed that these two types of inclusion bodies may have the same origin.

Microscopic examinations made of the animals in group 2, receiving very small quantities of lead arsenate, showed no abnormalities of definite significance.

Observations made on the animals of group 3, which had received 500 Gm. of diet containing 107.5 mg. of arsenic as arsenic trioxide, revealed a more or less generalized dilatation of capillaries in the viscera and parenchymatous degeneration of the liver. There were changes in the small intestine and distention of the cecum. The bone marrow was slightly hyperplastic.

The animals of group 4, receiving calcium arsenate, were significantly heavier than the controls, and autopsies showed large deposits of subcutaneous and abdominal fat. However, the increase in weight was entirely due to edema, since the average dry weight of a large number of animals from this same group used for chemical examination was almost the same as that of the controls.[3] There was marked edema of the subcutaneous and intra-abdominal fatty tissues and apparently also of the liver. The small intestine and the uterine horns were elongated, and the cecum was distended, which was probably due to muscular relaxation. There was generalized capillary dilatation, but the bone marrow and blood pictures were not markedly different from those of the controls. It is interesting that this group of animals showed much more marked signs of intoxication than did those of group 3 receiving arsenic at the same level. This is contrary to the usual conception that arsenic is more toxic in the trivalent form than in the pentavalent form.

Although a total of 29 dogs were used and observed post mortem, the protocols of only a few of them are presented. Of the 20 dogs receiving lead in their diet, 15 died before the termination of the experiment. All of the signs, symptoms and data from examinations indicated that no factor other than the lead included in their diet, even the very low level of 0.33 mg. of lead per kilogram of body weight per day,[4]

was involved in causing their death. Not 1 of the 7 dogs receiving diets to which no lead was added showed any signs of abnormality up to the time when they were put to death, at the termination of the experiment. Of the latter group, some were kept for over a year and had been raised from pups on the basic diet, diet B.

The kidneys of all the dogs which had been on diets containing lead up to the time of death showed tubular degeneration. The type of degeneration was different from that seen in the rats. In 3 dogs the bladder contained bloody urine, though the mucosa was intact. In all dogs which had had convulsions, hyperemia and edema of the brain were noted. The bones of many of the dogs receiving diets containing lead were hard, brittle and thickened, and in the long bones of this description the marrow cavities were constricted. Compensatory formation of marrow was noted in the skull. The lead deposits in the bones of the dogs resembled those seen in the rats. The dogs on lead whose blood was examined all had stippled red cells. One dog had nucleated red cells with basophilic stippling of the cytoplasm.

The pathologic investigations taken in conjunction with the extensive chemical investigations [4] indicate clearly that the ingestion of lead even at very low levels results in severe injury to the tissues of animals and ultimately in their death. Dogs 6 and 7, reported on here, received only 0.33 mg. of lead as lead acetate per kilogram of body weight per day. One of them died on the one hundred and fortieth day and the other on the one hundred and sixty-seventh day of the regimen. Five animals in this series received lead arsenate, and the results were the same as in the animals receiving lead acetate at the same level of lead in the diet. To make more certain of this, litter mates were used in some cases. The most striking differences were those due to age, younger animals being much more susceptible than older animals receiving the same level of lead.

SUMMARY

The microscopic observations reported in this paper bear out the chemical results reported elsewhere and show that under the conditions of our experiments injuries of tissues were caused by both lead and arsenic. The most marked histologic changes were produced in the kidney. The most severe injury and death resulted from the feeding of lead. Some dogs fed as little as 0.33 mg. of lead per kilogram of body weight per day died before the scheduled termination of the experiment. The manner of death of some of these animals corresponded in almost every particular with the convulsive terminations in cases of human lead poisoning described in the literature. In view of these results the conclusion is inescapable that lead and arsenic are chronic tissue poisons, the continued intake of which into the body is always fraught with the possible production of definite injury.

PATHOLOGIC EFFECTS OF CERTAIN GLYCOLS AND RELATED COMPOUNDS

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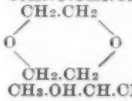
A number of polyhydric alcohols and their derivatives are being used in increasing quantities in many industries. They are used, for example, in the manufacture of lacquers, flavoring extracts, textiles, cosmetics, pharmaceuticals, preservatives, detergents and polishes. As part of a study of the toxicology of ethylene glycol (glycol), diethylene glycol (diglycol), propylene glycol and some of their derivatives, the organs of rats, rabbits and guinea pigs to which these substances had been administered were studied morphologically. The degenerative changes that occurred in some of these organs form the subject of this report.

TABLE 1.—*Chemical Formulas of Compounds Used* *

Compound	Structural Formula
Ethylene glycol; glycol	$HO.CH_2.CH_2.OH$
Ethylene glycol diacetate	$CH_3.CO.O.CH_2.CH_2.O.CO.CH_3$
Propylene glycol	$HO.CH_2.CH.OH.CH_3$
Diethylene glycol; diglycol	$HO.CH_2.CH_2.O.CH_2.CH_2.OH$
Ethyl diethylene glycol (diglycol monoethyl ether); carbitol	$C_2H_5.O.CH_2.CH_2.O.CH_2.CH_2.OH$
Methyl diethylene glycol (diglycol monomethyl ether); methyl carbitol	$CH_3.O.CH_2.CH_2.O.CH_2.CH_2.OH$
Butyl diethylene glycol (diglycol mono-n-butyl ether); butyl carbitol	$C_4H_9.O.CH_2.CH_2.O.CH_2.CH_2.OH$
Diethylene dioxide (dioxane)	
Dipropylene glycol	$CH_3.OH.CH.CH_2.O.CH_2.CH.OH.CH_3$

* The compounds were of commercial grade and were manufactured by the Carbide and Carbon Chemicals Corporation.

The chemical relationship of the several glycols used is indicated in table 1.

The compounds were given by mouth to rats and guinea pigs, usually in the drinking water, and by mouth or intravenously to rabbits, for from one to two hundred and thirty-four days. The animals were fed stock laboratory diets, and they were maintained in a state of good nutrition except as noted. The tissues of animals that died during the experiments or that were killed were promptly

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fixed in Zenker's solution, cut in paraffin and stained with hematoxylin and eosin. (The tissues of many animals dying during the night were discarded because of postmortem changes; none of these animals are included in this report.) Certain blocks were also fixed in formaldehyde for the study of fat and in absolute alcohol for the study of glycogen by Best's method.

The water content of the liver and kidneys was determined for certain of the animals by drying to constant weight at 90 C. The nonprotein nitrogen of the whole blood was determined by the micro-Kjeldahl technic for most of the animals that were killed at the end of an experiment.

ETHYLENE GLYCOL

The simplest member of the group of substances used is ethylene glycol, or, as it is often called, glycol. The effects of glycol have been extensively studied, and have been reviewed, together with the literature, by Browning.[1] Pathologically, they consist of deposition of masses of calcium oxalate in the renal tubules, with oxaluria and in some cases hematuria.

Eleven rabbits received from 1 to 4 cc. per kilogram of body weight of glycol intravenously in a single dose. They were killed at the end of from one to one hundred and twenty days. Four of the 11 presented the lesions to be described (as early as twenty hours after a dose of 4 cc. per kilogram) with a rise in the nonprotein nitrogen of the blood to 226 mg. in 100 cc.[2] Thirty rats received glycol in from 1 to 5 per cent concentration in the drinking water for from eight to seventy-nine days.[3] Thirteen died in from nine to fifty-one days, and 10 of them had lesions similar to those in the rabbits. Seventeen were killed at the expiration of from eight to seventy-nine days, and the kidneys of 10 were involved in the same way. Approximately 6 cc. of glycol per kilogram of body weight is required daily for nine days to cause renal lesions and death.

The renal lesions are characterized by slight enlargement of the kidneys, associated with many gray glistening streaks and dots in the cortex and medulla. These streaks consist of deposits of refractive, colorless or pale greenish granular and crystalline material in the tubules, chiefly the convoluted. Only a few tubules may be involved, or the deposits may be widespread. In the latter case, dilatation of the tubules proximal to the crystalline deposits may occur. In and about the plugged tubules a mild acute inflammatory reaction is sometimes set up. In some instances, a few epithelial cells are destroyed, and regeneration sets in from those remaining. The crystalline masses reduce silver nitrate and are soluble in 10 per cent nitric or sulfuric acid. They are composed at least in part of calcium oxalate.

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1. Browning, E.: Toxicity of Industrial Organic Solvents, Medical Research Council, Industrial Health Research Board, Report 80, London, His Majesty's Stationery Office, 1937.

2. The normal range of nonprotein nitrogen in the blood of control rats and rabbits is from 30 to 55 mg. in 100 cc.

3. Except when stated otherwise, the average daily intake of glycol water in these experiments was approximately 30 cc. per rat.

ETHYLENE GLYCOL DIACETATE

No previous study had been made of the effect on animals of the acetic acid ester of glycol.

Eleven rats received this chemical in from 1 to 5 per cent concentration in the drinking water for from seven to one hundred and thirty days. Four died in from seven to one hundred and fourteen days; lesions of the kidneys were present in all. The remaining 7 were killed at intervals of from fifteen to one hundred and thirty days; the kidneys of 4 were affected. The minimal dose required to produce damage in the kidneys was approximately 6 Gm. per kilogram daily, received in 5 per cent concentration, for seven days.

The kidneys of the animals affected contain deposits of calcium oxalate quite like those produced by glycol (fig. 1 *A*). Dilatation of the proximal portion of the convoluted tubules may also be present.

Further evidence of the composition of the crystalline deposits was obtained by analyzing the kidneys of 4 of the rats for calcium by the method of Halverson and Bergeim.[4] An unaffected kidney contained 9.2 mg. of calcium in 100 Gm. wet weight of kidney, an amount that is within the normal range. Three kidneys in which the crystalline deposits were found histologically contained 86.2, 104.1 and 819.2 mg. of calcium in 100 Gm. wet weight, respectively. The deposits were sufficiently large in the last animal to cause dilatation of the convoluted tubules proximally and slight nitrogen retention (blood nonprotein nitrogen —76.2 mg. in 100 cc.). However, no enlargement or hyperplasia of the parathyroid glands of any of these rats was demonstrable when the glands were sectioned serially. This is of interest in view of the well established sequence of renal insufficiency, hyperparathyroidism and increase in renal calcium.[5]

PROPYLENE GLYCOL

Of the several substances investigated, propylene glycol was the most innocuous. This fact has been emphasized by other investigators.[6] In vitro or when injected intravenously, it is hemolytic,[6a] but taken into the alimentary tract it is readily absorbed and rapidly oxidized. Lehman and Newman [6d] assigned to it a narcotic value of about one-third that of ethyl alcohol.

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4. Halverson and Bergeim, cited in Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry—Methods, Baltimore, Williams & Wilkins Company, 1932, p. 767.

5. Donohue, W.; Spingarn, C., and Pappenheimer, A. M.: J. Exper. Med. **66**:697, 1937.

6. (*a*) Hunt, R.: Indust. & Engin. Chem. **24**:836, 1932. (*b*) Seidenfeld, M. A., and Hanzlik, P. J.: J. Pharmacol. & Exper. Therap. **44**:109, 1932. (*c*) Hanzlik, P. J.; Mehrtens, H. G., and Spaulding, J. B.: ibid. **49**:300, 1933. (*d*) Lehman, A. J., and Newman, H. W.: ibid. **60**:312, 1937. (*e*) Holck, H. G. O.: J. A. M. A. **109**:1517, 1937.

EXPLANATION OF FIGURE 1

A, calcium oxalate crystals in convoluted tubules of the kidney of a rat which received ethylene glycol diacetate in a concentration of 1 per cent for one hundred and ten days and in a concentration of 3 per cent for twenty days, in the drinking water. The renal calcium amounted to 819 mg. in 100 Gm. wet weight; the blood nonprotein nitrogen, to 76.2 mg. in 100 cc.

B, hemoglobin casts in renal tubules of a rabbit given 5 cc. per kilogram of propylene glycol intravenously and killed on the ninth day. The hemoglobin content was 40 per cent (Dare); the blood nonprotein nitrogen, 69.4 mg. in 100 cc.

C, vacuolation of cells of convoluted tubules and slight leakage of protein into glomerular spaces in the kidney of a rabbit that received 2 cc. per kilogram of diethylene glycol intravenously and was killed twenty hours later.

D, hydropic degeneration of convoluted tubules and distention of glomerular spaces in the kidney of a rat that consumed diethylene glycol in a concentration of 5 per cent in the drinking water. The animal became sick and was killed at the end of seventy-two hours.

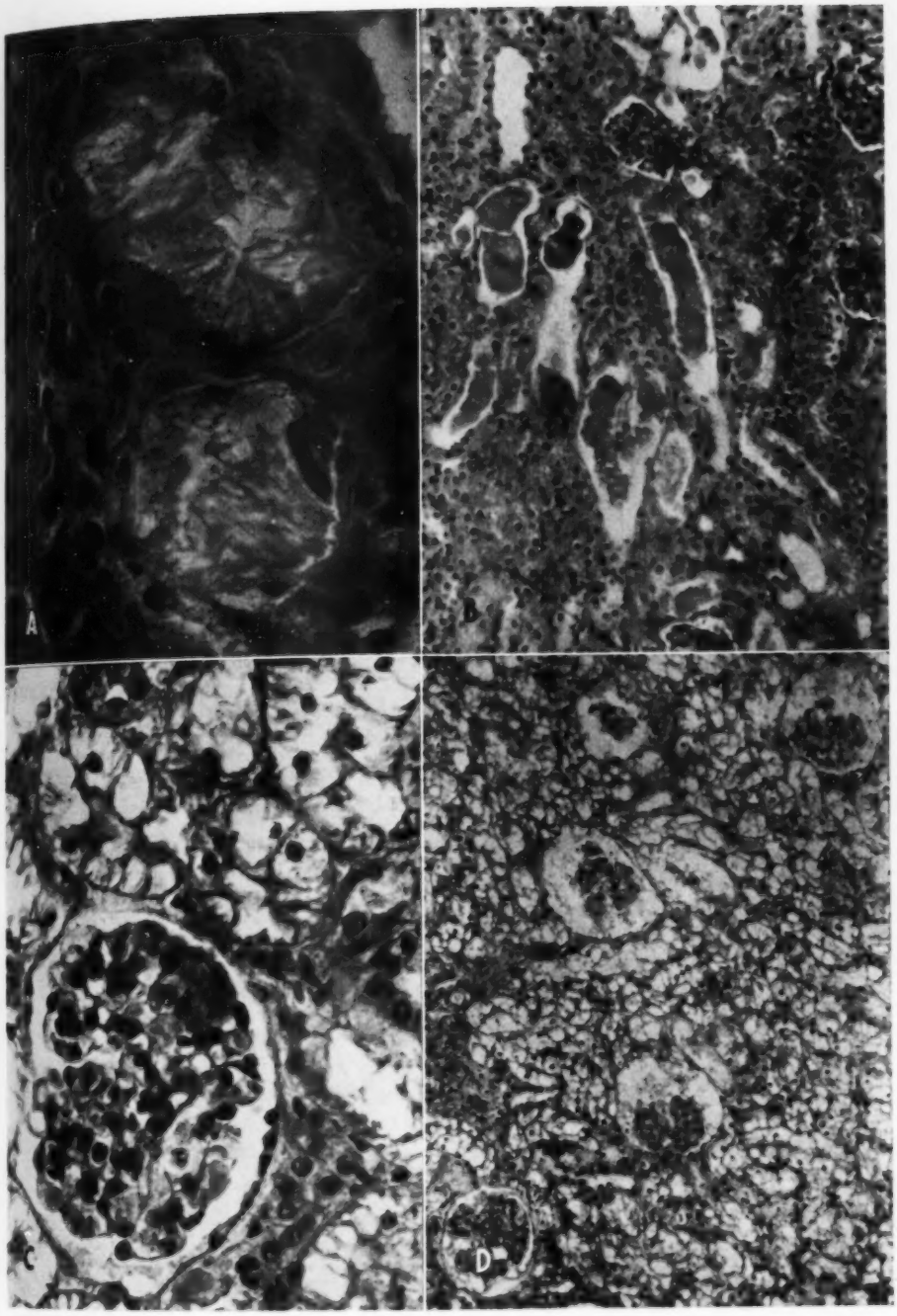

Figure 1

The 1,2 form of propylene glycol was administered to 39 rats in their drinking water in concentrations of from 1 to 10 per cent for from eleven to two hundred and thirty-four days with no demonstrable effect. Fifteen rabbits were given by intravenous injection single doses of from 2 to 5 cc. per kilogram of body weight. One rabbit that received a 5 cc. dose died the following night, but nothing abnormal was observed except the presence of considerable quantities of protein within the tubules of the kidneys. The other 14 rabbits were killed within from two to forty-six days after the injection. No pathologic changes were found in 10 of these.

Four rabbits which had been given intravenously 4 or 5 cc. of propylene glycol per kilogram of body weight and which were killed at the expiration of from two to twenty-three days exhibited in the kidneys evidence of the hemolytic action of the compound. This was visible grossly as reddish brown streaks and points on both the capsular and the cut surfaces. These discolorations consisted of hemoglobin-containing casts which could be found in all levels of the tubular system (fig. 1 *B*). Granular and hyaline casts were also sometimes present. Leakage of erythrocytes into glomerular spaces and convoluted tubules was sometimes seen. One of these rabbits which received a 5 cc. dose had 238 mg. of nonprotein nitrogen in 100 cc. of blood when killed forty-eight hours after the injection. In addition to all the aforementioned changes, small groups of renal convoluted tubules were necrotic, and occasional mitoses were found in adjacent remaining epithelial cells of this animal.

DIETHYLENE GLYCOL

Since the series of fatalities in October 1937 following the use of an elixir of sulfanilamide made with diethylene glycol as one of the solvents, several reports have appeared on the pathologic effects of large doses of diglycol on human beings and animals (Kesten, Mulinos and Pomerantz;[7] Holck;[6e] Cannon;[8] Ruprecht and Nelson;[9] Hagebusch;[10] Lynch;[11] Cannon and Geiling[12]). Prior to that time only two studies of the toxicity of diglycol had been published (von Oettingen and Jirouch;[13] Haag and Ambrose[14]), in neither of which were details of pathologic changes noted. Von Oettingen and Jirouch[13] mentioned

7. Kesten, H. D.; Mulinos, M. G., and Pomerantz, L.: J. A. M. A. **109**: 1509, 1937.
8. Cannon, P. R.: J. A. M. A. **109**:1536, 1937.
9. Ruprecht, H. A., and Nelson, I. A.: J. A. M. A. **109**:1537, 1937.
10. Hagebusch, O. E.: J. A. M. A. **109**:1537, 1937.
11. Lynch, K. M.: South. M. J. **31**:134, 1938.
12. Cannon, P. R., and Geiling, E. M. K.: J. A. M. A. **111**:919, 1938.
13. von Oettingen, W. F., and Jirouch, E. A.: J. Pharmacol. & Exper. Therap. **42**:355, 1931.
14. Haag, H. B., and Ambrose, A. M.: J. Pharmacol. & Exper. Therap. **59**: 93, 1937.

the occurrence of acute nephrosis in rats that had been given subcutaneous injections of one or the other of the following: diethylene glycol, its ethyl ether, or carbitol; the ethyl ether of ethylene glycol, or cellosolve; cellosolve acetate; butyl cellosolve, and dioxane.

The effects of diglycol were studied as follows:

1. Seventeen rats received diglycol in 0.5 per cent concentration in the drinking water for from twenty-two to one hundred and twenty-four days. No deaths and no lesions occurred as a result of the procedure.

2. Thirty rats drank 1 per cent diglycol for from thirty-three to two hundred and seventeen days without any demonstrable effect.

3. Thirty rats received 3 per cent diglycol in the drinking water for from four to ninety-five days (approximately 3.5 cc. of diglycol per kilogram of body weight per day). Twenty-one died in from four to fifty-six days; hydropic degeneration of the renal cortex was present in 20. The livers of 13 of these contained foci of similar degeneration. The remaining 9 were killed at from fifty-one to ninety-five days. No lesions referable to the procedure were present.

4. Forty-two rats were on 5 per cent diglycol (approximately 6 cc. per kilogram of body weight per day) for from one to thirty-six days, of which 9 died in from one to six days. Hydropic degeneration had occurred in the kidneys of the 9 animals and in the livers of 5. Of the remaining 33, killed at from one to thirty-six days, similar damage of the kidneys was exhibited in 27 and degeneration of the liver in 13.

5. Six rabbits received from 1 to 4.6 cc. of diethylene glycol per kilogram of body weight in from 1 to 5 per cent concentration in the drinking water during from five to twenty-eight days. One rabbit which had consumed 1 cc. per kilogram died on the seventh day with extensive renal damage and edema of the lungs. Of 5 killed at from five to twenty-eight days, 4 presented lesions in the kidneys and 2 in the liver.

6. Thirty-five rabbits were given intravenous injections of from 1 to 2 cc. per kilogram of body weight, usually in a single dose, of full strength or 50 per cent diglycol (redistilled in a few instances). Eleven animals died in from one and a half to twenty-one days. The kidneys of 10 and the livers of 8 of them had undergone varying degrees of hydropic degeneration. The remaining 24 were killed after from one to forty-four days, and similar changes were observed in the kidneys of 15, and in the livers of 9. One instance was noted in which, following the injection of 2 cc. per kilogram of body weight, twenty hours was sufficient for the development of extensive changes in the kidneys, with a rise in the nonprotein nitrogen of the blood to 86 mg. in 100 cc. (fig. 1 *C*).

7. Five guinea pigs received from 2 to 5 cc. per kilogram of body weight of diglycol by mouth during from two to twelve days. Two died at three and five days, respectively, with lesions in the kidneys similar to those in the rats and rabbits. One showed the liver similarly involved. The remaining 3 were killed at from two to twelve days. Hydropic degeneration had occurred in the kidneys of all and in the livers of 2.

Control animals on the same stock diet without the addition of diglycol remained normal.

Animals affected by diglycol become comatose and often anuric prior to death. For several hours following intravenous injection of diglycol

EXPLANATION OF FIGURE 2

A, calcification and regeneration of the convoluted tubules in the kidney of a rabbit that received 1 cc. per kilogram of diethylene glycol intravenously. The animal became sick and was killed on the ninth day. The blood nonprotein nitrogen amounted to 389 mg. in 100 cc.

B, hydropic degeneration of liver cells about efferent veins in a rabbit that received 2 cc. per kilogram of diethylene glycol intravenously. Death occurred in thirty-six hours.

C, hydropic degeneration of convoluted tubules of the kidney of a rat the drinking water of which contained 5 per cent methyl carbitol for twenty-eight days.

D, hydropic degeneration of convoluted tubules of the kidney of a rat which received butyl carbitol in a concentration of 5 per cent in the drinking water. It was killed on the eighth day. The blood nonprotein nitrogen was 162 mg. in 100 cc.

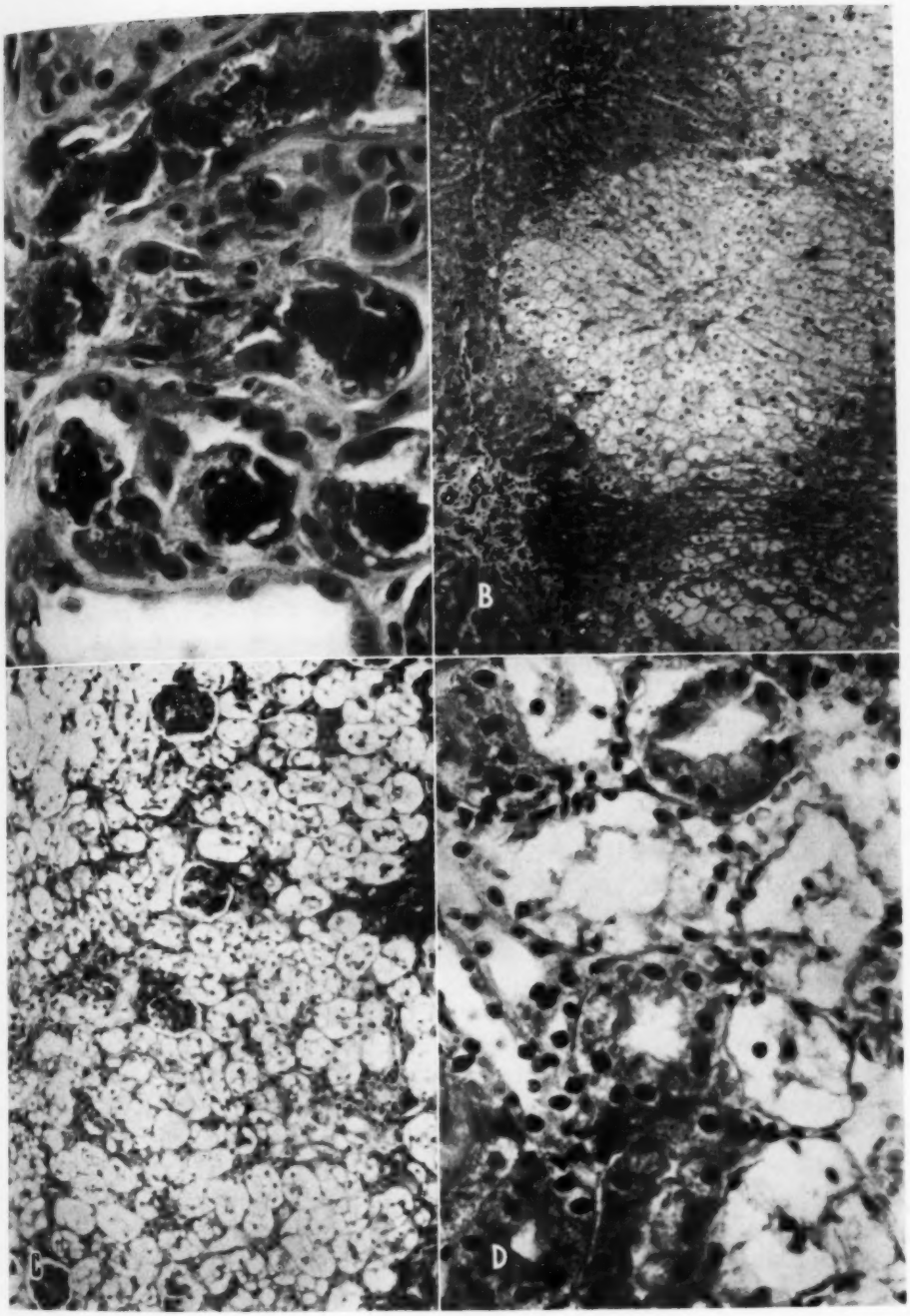

Figure 2

many of the rabbits pass smoky urine which reacts with benzidine. The nonprotein nitrogen of the blood in all the affected animals mounts steadily to from eight to ten times the normal amount (e. g., 389 mg. in 100 cc. in a rabbit that lived nine days after the injection of 1 cc. per kilogram of body weight).

The organs regularly affected in diglycol poisoning are the liver and kidney. The kidneys of the three species of animals used are swollen, pale yellowish tan and apparently more moist than normal. On analysis the water content is found to be increased approximately 6 per cent (to an average of 84 per cent from a normal average of 78 per cent). The cortical markings are indistinct. The medulla often appears to be congested. The outstanding histologic change is hydropic degeneration of the convoluted tubules. Every tubule may be and commonly is involved, or the change may be confined to isolated small groups of tubules. The epithelial cells swell and undergo coarse vacuolation, often with fusion of the vacuoles to a single large space that represents the entire cell save for an irregular cell membrane and a compressed pyknotic nucleus. The vacuoles are devoid of fat or glycogen, but occasionally a little finely divided fat is present in the remaining cytoplasm or in otherwise unchanged epithelial cells. Necrosis of the vacuolated cells occurs, and the tubular lumen becomes blocked by swollen cells and cell detritus. Obstruction to the outflow of urine ensues, leading to dilatation of the glomerular spaces. The glomerular tufts are often compressed and bloodless but otherwise are essentially uninvolved. Calcification of necrotic cytoplasm may set in as early as the fifth day in rabbits, and attempts at regeneration may be present, characterized by mitoses (fig. 2 *A*). Leukocytic reaction is not a feature. Changes in the medulla are confined to congestion of the capillaries and small veins and to the occurrence of many hyaline and granular casts in the collecting tubules. Occasionally a little finely divided fat is found in the epithelium of such tubules.

The liver, though less frequently involved, may be enlarged and pale, and appear moist. An increase of approximately 10 per cent in volatile matter is observed (to an average of 82 per cent from a normal average of 72 per cent). The cells about the efferent vein in each lobule, often extending well out toward the portal areas, enlarge, and the cytoplasm becomes pale. Many tiny vacuoles appear in it, which fuse together until the cell consists of a single large vacuole surrounding a compressed and pyknotic central nucleus (fig. 2 *B*). These vacuoles are also free from fat and glycogen, but in rare instances a little finely divided stainable fat is present in the other liver cells.

In a small percentage of the animals that have changes in the kidneys and liver, the epithelial cells of the outer fourth of the adrenal cortex

are swollen, pale staining and vacuolated in the same manner as the liver cells. The vacuoles do not contain stainable fat.

The lesions noted in the kidney and liver are essentially similar to those which have been described by other investigators [15] coincident with and since the appearance of our preliminary report.[7] The kidneys of animals, however, apparently do not contain the small arterial and capillary thromboses and massive cortical necrosis found in the kidneys of some of the human beings who were poisoned by large quantities of diglycol.[16]

ETHYL DIETHYLENE GLYCOL (CARBITOL)

Three ether derivatives of diethylene glycol were investigated briefly: ethyl diethylene glycol (diglycol monoethyl ether), or carbitol; methyl diethylene glycol (diglycol monomethyl ether), or methyl carbitol, and butyl diethylene glycol (diglycol mono-n-butyl ether), or butyl carbitol.

Carbitol is among the chemicals reported by von Oettingen and Jirouch [13] as producing acute nephrosis in rats. A communication from the director of a pharmaceutical laboratory to the Council on Pharmacy and Chemistry of the American Medical Association [17] stated that the equivalent of 1.67 cc. per kilogram of body weight of carbitol had been administered to rats daily for twenty days without apparent effect. One rat of the series which was put to death after sixteen days had normal kidneys and liver.

The drinking water of 6 rats was made up to 5 per cent concentration of carbitol, and the rats consumed varying amounts of it for from one to fifteen days. The intake was not over half the intake of drinking water in the preceding experiments, averaging approximately 15 cc. per day. Two rats died on the third and fifth days, respectively, and exhibited evidence of renal disease. Of the remaining 4, which were killed at the expiration of from one to fifteen days, 2, killed on the fifth day, had a nonprotein nitrogen concentration in the blood of 279 and 289 mg. in 100 cc., respectively, and exhibited lesions of the kidneys. Because of the difficulty encountered in obtaining an adequate intake of the material in the drinking water, an attempt was made to administer a solution to 2 rats by stomach tube, but this led to acute bronchitis and bronchopneumonia with death on the second and third days, respectively, without renal involvement.

A total of approximately 10 cc. of carbitol per kilogram of body weight had been consumed in the drinking water by the rat that died on the third day and that had lesions in the kidneys, and 15 cc. per kilogram by the one that died on the fifth day. However, the intake of fluid by the rats to which carbitol was offered was below normal, so that partial dehydration may have contributed to the result. Furthermore, many of the animals receiving carbitol,

15. Cannon.[8] Ruprecht and Nelson.[9] Hagebusch.[10] Lynch.[11] Cannon and Geiling.[12]

16. Cannon.[8] Ruprecht and Nelson.[9] Lynch.[11] Cannon and Geiling.[12]

17. "Carbitol," Queries and Minor Notes, J. A. M. A. **110**:1692, 1938.

methyl carbitol and butyl carbitol refused food for some time before death, and a certain degree of starvation was commonly present. Such animals not infrequently showed pathologic changes associated with inanition, such as excessive hemosiderin deposits in the spleen, small hemorrhages and erosions in the mucous membrane of the alimentary tract, and enlargement of the adrenals.[18]

The kidneys of rats receiving carbitol undergo changes identical in every respect with those seen in animals on diethylene glycol itself, i. e., extensive hydropic degeneration of the epithelium of the convoluted tubules leading to obstruction. The other viscera examined were essentially normal.

METHYL DIETHYLENE GLYCOL (METHYL CARBITOL)

No experiments on the toxicity of this compound had been carried out previously.

Nine rats were given methyl carbitol in from 3 to 5 per cent concentration in their drinking water for from eleven to sixty-four days. (Four of these had previously been on 1 per cent methyl carbitol for one hundred and ten days without apparent effect.) One animal died, at the end of sixty-four days. Except for moderate amounts of protein in the lumens of the convoluted tubules of the kidney, its organs were normal. Of the 8 that were killed at the expiration of from eleven to forty-five days, the kidneys of 3 (twenty-eight, twenty-eight and forty-five days, respectively) had been damaged.

Methyl carbitol is also not well taken in the drinking water by rats. Two attempts to administer the compound by stomach tube were followed by acute bronchitis and pneumonia.

Methyl carbitol, ingested by mouth, produces damage in the kidneys of rats analogous to that produced by diglycol (fig. 2 *C*). Somewhat larger amounts of the methyl derivative than of carbitol itself are apparently necessary to produce these effects.

BUTYL DIETHYLENE GLYCOL (BUTYL CARBITOL)

Browning [1] refers to a factory worker exposed to either butyl carbitol or butyl cellosolve or both, in whom hematuria with casts developed. No animal experiments on the effects of butyl carbitol had been carried out previously.

The compound was administered in from 3 to 5 per cent solution in the drinking water of 9 rats for from five to thirty-five days. (Two of these animals had previously been on 1 per cent butyl carbitol for one hundred and nine days without apparent effect.) Three rats died on the fifth, twenty-third and thirty-fifth days, respectively. The kidney of the rat that died on the fifth day contained an occasional small group of damaged convoluted tubules. The kidneys of 2 of the remaining rats, killed at the expiration of from eight to

18. Jackson, C. M.: Effects of Inanition and Malnutrition upon Growth and Structure, Philadelphia, P. Blakiston's Son & Co., 1925.

sixteen days, had undergone similar changes. Butyl carbitol in from 3 to 5 per cent concentration, is relatively unpalatable to rats, leading to partial dehydration and partial starvation.

The renal lesions caused by this compound are similar to those following the administration of diethylene glycol but are much less extensive with the dosage used (fig. 2 *D*).

DIETHYLENE DIOXIDE (DIOXANE)

Dioxane, the second ether of ethylene glycol, contains two ether linkages in its molecule. It is a widely used solvent, and its toxicity has been extensively studied. The literature has been summarized by Browning.[1] Dioxane is in the series of compounds causing acute nephrosis studied by von Oettingen and Jirouch.[13] Fairley, Linton and Ford-Moore[19] described peripheral necrosis of hepatic lobules and necrosis of tubular epithelium in the kidneys of guinea pigs, rats and rabbits receiving the substance, although Yant, Schrenk, Waite and Patty[20] in an earlier series of acute inhalation experiments found no lesions of these organs in guinea pigs. Following a report by Barber[21] of the death of 5 workers in the manufacture of artificial silk all of whom had central necrosis of the liver and symmetric necrosis of the renal cortex associated with vascular lesions, De Navasquez[22] reinvestigated the effects of dioxane when given intravenously and intragastrically to rabbits, cats and guinea pigs. He observed acute hydropic degeneration of the convoluted tubules in the kidneys of all the animals, and in rabbits, vacuolation of the liver cells about the efferent veins, the vacuoles being apparently filled with glycogen.

Dioxane is highly toxic to rats. Of 10 animals given the compound in 5 per cent concentration in their drinking water, 8 died in from five to twelve days. The kidneys of the 8 rats were the site of extensive degeneration, and the livers of 5 were also abnormal. The kidneys of the remaining 2 animals, killed on the sixth and eighth days, respectively, were similarly involved. One of these also showed involvement of the liver. Two rats on 1 per cent dioxane for one hundred and ten days, followed by 3 per cent for forty-one and forty-eight days, respectively, had patchy areas of degeneration in the kidneys. One also had hepatic damage.

Five per cent dioxane is relatively unpalatable to rats and was poorly taken. The estimated intake of dioxane was 1 cc. per kilogram of body weight per day, which sufficed to cause fatal renal damage in five days—an observation which emphasizes the relatively high toxicity of this compound when taken by mouth.

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19. Fairley, A.; Linton, E. C., and Ford-Moore, A. H.: J. Hyg. **34**:486, 1934.
20. Yant, W. P.; Schrenk, H. H.; Waite, C. P., and Patty, F. A.: Pub. Health Rep. **45**:2023, 1930.
21. Barber, H.: Guy's Hosp. Rep. **84**:267, 1934.
22. De Navasquez, S.: J. Hyg. **35**:540, 1936.

EXPLANATION OF FIGURE 3

A, hydropic degeneration of the renal cortex in a rat which received dioxane in a concentration of 5 per cent in the drinking water. Death occurred on the sixth day.

B, hydropic degeneration of liver cells about efferent veins in a rabbit which received 1.5 cc. per kilogram of dioxane intravenously and was killed on the fourth day. The blood nonprotein nitrogen amounted to 242 mg. in 100 cc.

C, hydropic degeneration of the renal cortex of a rabbit that received 4 cc. per kilogram of dipropylene glycol and was killed seventy-two hours later. The blood nonprotein nitrogen amounted to 286 mg. in 100 cc.

D, hydropic degeneration of the liver cells adjacent to an efferent vein in a rabbit that received 4 cc. per kilogram of dipropylene glycol intravenously and was killed at the expiration of seventy-two hours.

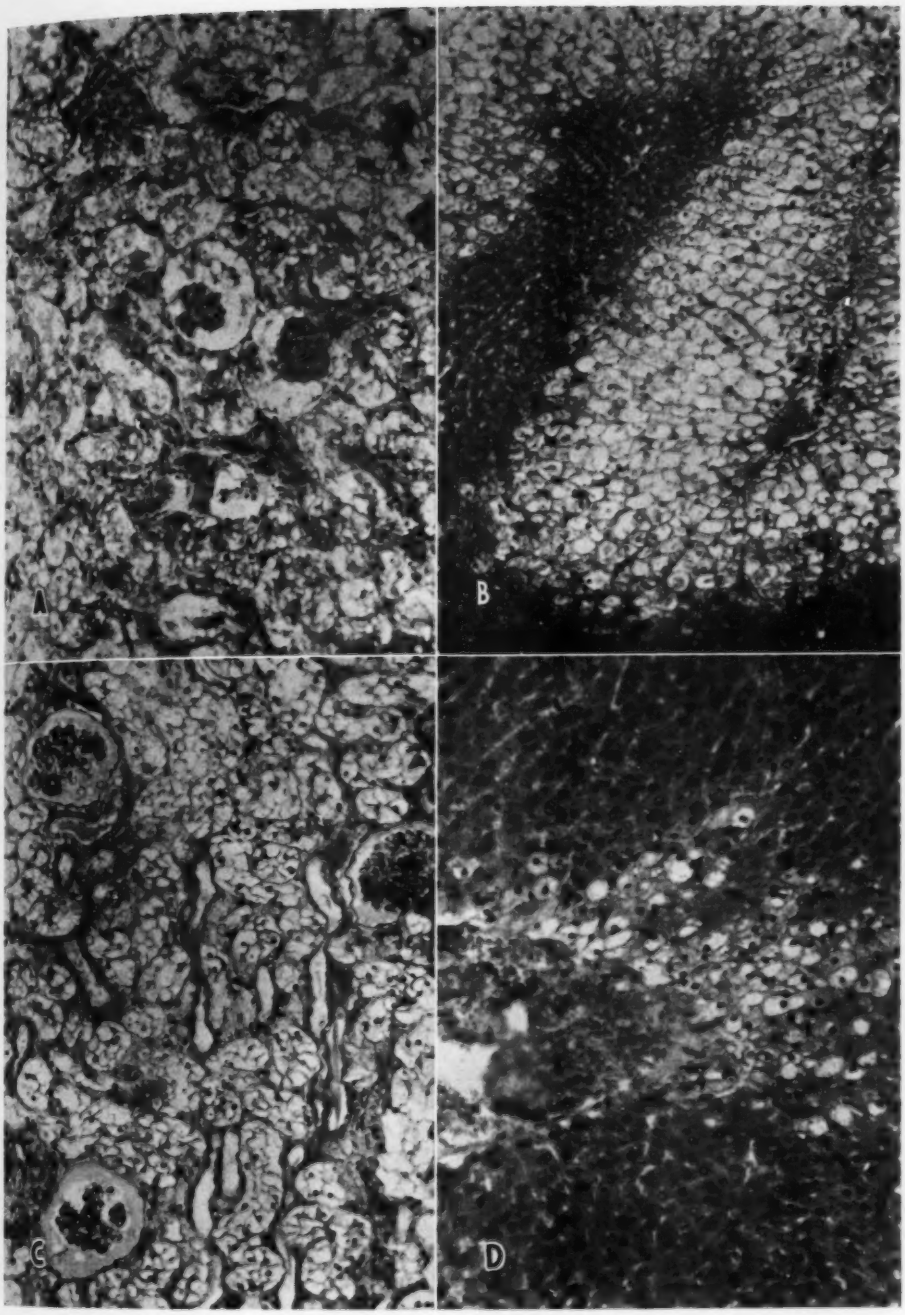

Figure 3

Two rabbits were given intravenous injections of 1.5 cc. of dioxane per kilogram of body weight in 25 per cent solution. One died two days later, and its kidneys exhibited several cortical areas of degeneration. The kidneys and liver of the second rabbit, killed on the fourth day, were markedly damaged, and the nonprotein nitrogen of the blood had risen to 242 mg. in 100 cc.

The pathologic observations in the kidneys of the animals are essentially those described by De Navasquez [22] and by Fairley, Linton and Ford-Moore.[19] They reproduce exactly the lesions due to diethylene glycol, namely, extensive hydropic degeneration of the epithelium of the convoluted tubules, every tubule being involved in most of the animals (fig. 3 *A*). High levels of blood nonprotein nitrogen ensue. Symmetric cortical necroses are not seen.

The livers of affected animals are also the site of the same lesion observed in the animals receiving diglycol, namely, swelling and hydropic degeneration of the liver cells about efferent veins (fig. 3 *B*). De Navasquez found the vacuoles in the liver cells to be filled with glycogen and was doubtful whether they could be considered pathologic. Although we were able to stain a little glycogen in some of the liver cells (by fixing thin slices in absolute alcohol immediately after death, embedding in pyroxylin, and treating with Best's carmine stain), this glycogen is finely divided or in a diffuse state and is in the cytoplasm present about the vacuoles. The vacuoles themselves remain empty looking and unstained. Fat is not present in them, nor is it present in the vacuolated cells in the kidney. A little finely divided fat is, however, occasionally seen in both the epithelial and the Kupffer cells of the liver.

DIPROPYLENE GLYCOL

The pathologic effects of dipropylene glycol, the next higher homologue of diethylene glycol, had not been studied previously.

The drinking water of 7 rats contained from 1 to 5 per cent of dipropylene glycol for from thirty-three to seventy-seven days, without detectable effect on the animals. All organs were normal. When, however, a concentration of 10 per cent was given over periods of from nine to sixty-eight days to a total of 25 rats, 7 died at the end of from ten to thirty days. The kidneys of 5 of the dead rats had become the site of lesions (as early as the tenth day in 1 rat). The remaining 18 rats were killed after from nine to sixty-eight days. The kidneys of only 4 of these had become involved (as early as the ninth day in 1).

Ten rabbits received from 2 to 4 cc. per kilogram of body weight of dipropylene glycol intravenously. Two of these rabbits died on the fourth day, and lesions had occurred in the kidneys of both. The remaining 8 were killed after from one to twenty-one days. The kidneys of 3 exhibited similar lesions (as early as the third day in a single instance). One of these also had involvement of the liver. A dose of 4 cc. per kilogram was necessary to cause renal changes and to raise the nonprotein nitrogen of the blood (to as high as 406 mg. in 100 cc. on the sixth day in 1 rat).

Lesions identical to those produced by diethylene glycol are present in animals receiving dipropylene glycol. Extensive hydropic degeneration of renal epithelium is seen, with, in some cases, an occasional hemoglobin-containing cast in the collecting tubules (fig. 3 *C*). Damage to liver parenchyma is more inconstant and more difficult to produce (fig. 3 *D*).

TABLE 2.—*Summary of Protocols*

Compound	Animals	Route of Administration	Amount	Duration, Days	Observations in Kidney (Nature of Lesion; Percentage of Animals Affected)	Observations in Liver (Hydropic Degeneration; Percentage of Animals Affected)
					Oxalate crystals	
Ethylene glycol; glycol...	30 rats	Mouth	1-5%	8-79	67	0
	11 rabbits	Vein	1-4 cc. per Kg.	1-120	36	0
Ethylene glycol diacetate	11 rats	Mouth	1-5%	7-130	73	0
					Hemoglobin casts	
Propylene glycol.....	39 rats	Mouth	1-10%	11-234	0	0
	15 rabbits	Vein	2-5 cc. per Kg.	1-46	27	0
					Hydropic degeneration	
Diethylene glycol; diglycol	47 rats	Mouth	0.5-1%	22-217	0	0
	72 rats	Mouth	3-5%	1-95	78	43
	6 rabbits	Mouth	1-5%	5-28	83	33
	35 rabbits	Vein	1-2 cc. per Kg.	1-44	71	49
	5 guinea pigs	Mouth	2-5 cc. per Kg.	2-12	100	60
Ethyl diethylene glycol (diglycol monoethyl ether); carbitol	6 rats	Mouth	5%	1-15	67	0
Methyl diethylene glycol (diglycol monomethyl ether); methyl carbitol	9 rats	Mouth	3-5%	11-64	33	0
Butyl diethylene glycol (diglycol mono-n-butyl ether); butyl carbitol	9 rats	Mouth	3-5%	5-35	33	0
Diethylene dioxide; dioxane	10 rats	Mouth	5%	5-12	100	60
	2 rabbits	Vein	1.5 cc. per Kg.	2-4	100	50
Dipropylene glycol.....	7 rats	Mouth	1-5%	33-77	0	0
	25 rats	Mouth	10%	9-68	36	0
	10 rabbits	Vein	2-4 cc. per Kg.	1-21	50	10

COMMENT

The several experiments are summarized in table 2. From them there emerge the following observations: (*a*) ethylene glycol and ethylene glycol diacetate may cause deposition of oxalates in the kidneys and mild renal insufficiency; (*b*) propylene glycol is relatively harmless, especially when given by mouth, large intravenous doses being necessary to cause hemoglobinuria and casts; (*c*) diethylene glycol, the methyl, ethyl and butyl ethers of diethylene glycol, dioxane and dipropylene glycol are all capable of causing profound degeneration of renal epithelium leading to uremia.

The members of this last group of compounds have at least one structural factor in common. The molecules of diglycol and its ethers, of dipropylene glycol and of dioxane contain an ether linkage between two glycol molecules. The cyclic compound dioxane possesses two such linkages, which may in part account for its relatively high toxicity. The molecules of ethylene glycol, ethylene glycol diacetate and propylene glycol lack this particular type of ether linkage. At the same time their respective effects on parenchymatous epithelium differ from those of the preceding compounds. It is suggested that the ether linkage between two glycol molecules may be responsible for the hydropic degeneration of the epithelium of the convoluted tubules and of the liver cords.

An outstanding feature of the effect of the compounds causing hydropic degeneration of the kidney is the extensive character of the damage wrought. In each experiment one or more of the animals was apparently unaffected by the drug, but in the injured animals the lesions were usually maximal, involving as a rule the entire cytoplasm of every cell in every convoluted tubule. Occasionally only groups of tubules were the site of degeneration, but almost invariably every cell of the segment of tubule involved was completely hydropic. Multiple small vacuoles in a given cell were uncommon. It would seem that the colloidal structure of the cytoplasm of an injured cell relatively suddenly loses its integrity and approximates a true solution.

The animals that received diglycol and its ethers, dioxane and dipropylene glycol died usually as a result of the damage to the convoluted tubules, in uremia. The carbitols and dipropylene glycol are not palatable to rats in moderate concentrations, so that some of these animals consumed less water than their minimal requirements in view of the dry nature of their diet. Partial refusal of food sometimes followed, and dehydration and starvation were probably factors in the death of certain of these animals. The cause of death of the animals that received glycol and glycol diacetate was not always obvious. In not all instances were the deposits of oxalates in the kidney sufficiently extensive to cause demonstrable obstruction of tubules and a significant rise in the nonprotein nitrogen of the blood. As suggested by Hunt[6a] a question arises as to the possible formation of toxic intermediary products.

The relative toxicity of these compounds is roughly indicated in the protocols and will be taken up in detail in a separate report. It may be emphasized, however, that appreciable amounts of the several chemicals must be given to produce demonstrable changes in the animals used. In no instance was less than 1 cc. per kilogram of body weight effective.

CONCLUSIONS

Dipropylene glycol, diethylene glycol, dioxane, carbitol, methyl carbitol and butyl carbitol when administered in adequate dosage orally or intravenously to animals cause extensive hydropic degeneration of the renal convoluted tubules, leading to uremia; less regularly, there is hydropic degeneration of the liver parenchyma. These compounds resemble each other in containing an ether linkage between glycol molecules. Ethylene glycol, ethylene glycol diacetate, and propylene glycol do not have such a structure, nor do they cause similar lesions in animals.

CARTILAGINOUS FOCI IN THE HEARTS OF WHITE RATS AND OF MICE

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Spontaneous cardiovascular lesions in small rodents used for experimental purposes have been reported recently (Hueper [1]; Wilens and Sproul [2]). No mention has been made of cartilage in the hearts of rats and mice as described in this report.

During routine histologic examination of several thousand hearts of white rats and mice small cartilaginous nodules or bands in the region of the aortic ring were observed in a number of animals, which were as a rule young adults. As no systematic study was made, accurate figures for the incidence of the cartilagenous formations and for their relation to age are not available. The hearts were usually cut longitudinally through the base and apex. The presence or absence of the aortic bulb in the sections determined mainly the chance of finding cartilaginous areas. They were seen in about 100 of approximately 3,000 rats and in about 15 of 1,000 mice. The actual incidence probably was much higher.

There are usually two cartilaginous nodules in the aortic ring, one at the site of attachment of the right semilunar valve and the other at the base of the left semilunar valve. It is not rare that a sort of transitional tissue or even well developed cartilage connects the two nodes in the form of a curved band. The nodules are irregular in shape and indistinctly defined. In their central portions they consist of a hyaline cartilage, while in the marginal parts they contain some fibrillar material, form small cartilaginous buds and gradually merge with the surrounding fibroblastic, myxomatoid or muscular tissue. The cartilaginous cells are embedded in a bluish-staining matrix. They are often vesicular or seem to have undergone mucoid transformation. As shown in the figure, the cells contain not infrequently two oval or round nuclei, located in small cavities.

It is obvious that these cartilaginous deposits in the fibrous skeleton of the heart are not the products of a metaplastic process, such as that observed occasionally in degenerative foci of the heart and arteries of

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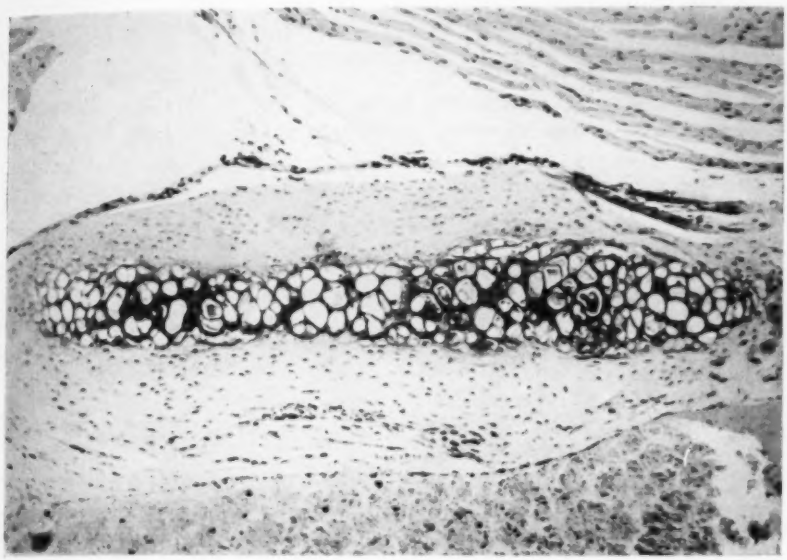
From the Warner Institute for Therapeutic Research.

1. Hueper, W. C.: Arch. Path. **20**:708, 1935.
2. Wilens, S. L., and Sproul, E. E.: Am. J. Path. **14**:177 and 201, 1938.

man and animals (Ribbert [3]; Mönckeberg [4]; Weizmann [5]; Spiegel [6]; Miesowicz [7]; Otto [8]; Lillie [9]) but are the results of a normoplastic transformation of fibrous tissue exposed to special stress under physiologic conditions.

These cartilaginous foci are comparable to similar ones in the hearts of mammals (horse, sheep, buffalo, rabbit), reptiles (turtle, alligator, crocodile) and birds (chicken) (Retterer and Lelièvre [10]; Kern [11]; Hausotter [12]; Favaro [13]; Greil [14]; Beddard and Mitchell [15]; Stiefel [16];



Cartilaginous plate in the aortic ring of the heart of a rat.

3. Ribbert, H.: Lehrbuch der allgemeinen Pathologie und der vergleichenden Anatomie, Leipzig, F. C. W. Vogel, 1923.
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11. Kern, A.: Morphol. Jahrb. **58**:125, 1927.
12. Hausotter, E.: Wien. tierärztl. Monatschr. **11**:311, 1924.
13. Favaro, G.: Atti mem. r. Accad. di sc., lett. ed arti **28**:2, 1912.
14. Greil, A.: Morphol. Jahrb. **31**:125, 1903.
15. Beddard and Mitchell, cited by Greil.[14]
16. Stiefel, K.: Anat. Anz. **61**:177, 1926.

Benninghoff [17]). I can confirm by personal observations the report that such nodules occur in the hearts of rabbits (Vanzetti [18]), studied during the same period as the rats and mice. In all instances the cartilage is hyaline. In sheep it may change into bone with increasing age through enchondral ossification. In birds it has been found not only in the aortic ring but also to a minor extent in the annulus of the pulmonary artery.

SUMMARY

Attention is called to the not infrequent occurrence of small cartilaginous areas in the aortic ring in white rats and mice which are not pathologic lesions but physiologic formations.

17. Benninghoff, A.: Das Herz, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1930, vol. 6, pt. 1, p. 187.

18. Vanzetti, F.: Arch. ital. de biol. **56**:265, 1911.

SPIROCHETES IN THE GASTRIC GLANDS OF MACACUS RHESUS AND OF MAN WITHOUT RELATED DISEASE

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The observation made by Cowdry and Scott [1] that spirochetes occur in the gastric glands of normal-appearing monkeys of the species Macacus rhesus is of interest, for these animals are closer to man than dogs, cats and rats, in which spirochetes have been reported to occur in the same location, without any accompanying signs of illness.[2] For reasons difficult to explain, these spirochetes can be easily seen in ordinary sections stained with hematoxylin and eosin.

Data on spirochetes in the human stomach are less definite than data on spirochetes in the stomachs of animals. Escherich [3] described spirochetes in the feces of patients with cholera and his observations have been confirmed. Le Dantec [4] called attention to their occurrence in the feces of patients with diarrhea of long standing, which he did not believe to be cholera. With the introduction of arsenicals specific for syphilis, the diagnosis of spirillar dysentery was established.

Spirochetes in the human stomach were first reported by Salomon [2b] in 1896. Confirmation followed, and it was emphasized that spirochetes were seen in the human stomach only in the presence of gastric carcinoma and only when the carcinoma was ulcerating. Their number was said to be proportional to the extent of the ulceration. Hoffmann [5] stated that the spirochetes do not enter the tissue but are limited to the necrotic material at the surface. They were found by Krienitz [6] in gastric contents removed by evacuation. Attempts to establish an etiologic relation between spirochetes and non-neoplastic gastric ulcers have not been successful. Some investigators have gone so far as to state that spirochetes are never found in them.[7] Luger [8] explained the presence of spirochetes in ulcerating carcinomatous areas and their absence in ulcers as due to the liberation in the former of proteins and amino acids which stimulate their growth.

From the Anatomical Laboratory, Washington University School of Medicine.

1. Cowdry, E. V., and Scott, G. H.: Arch. Path. **22**:1, 1936.

2. (*a*) Bizzozero, G.: Arch. f. mikr. Anat. **42**:82, 1893. (*b*) Salomon, H.: Centralbl. f. Bakt. (Abt. 1) **19**:433, 1896.

3. Escherich, T.: München. med. Wchnschr. **15**:408, 1884.

4. Le Dantec: Compt. rend. Soc. de biol. **55**:617, 1903.

5. Hoffmann: Berl. klin. Wchnschr. **28**:550, 1905.

6. Krienitz, W.: Deutsche med. Wchnschr. **22**:872, 1906.

7. Celler, H. L., and Thalheimer, W.: J. Exper. Med. **23**:791, 1916. Rosenow, E. C., and Stanford, A. H.: J. Infect. Dis. **17**:219, 1915.

8. Luger, A.: Wien. klin. Wchnschr. **52**:1643, 1917; Ztschr. f. klin. Med. **92**:54, 1921.

As to the nature or source of the spirochetes, little is known. Macfie and Carter [9] described their occurrence in the stools of 56.2 per cent of normal persons. Whether those in the stools and stomach are identical is not known; but the consensus seems to be that they are neither the spirochetes of Vincent's angina nor those of syphilis.

Investigations on animals and on man have been but inadequately correlated. To obtain material from normal persons is possible only in cases of accidental death. In such cases tissues have apparently not been examined for spirochetes. In a preliminary note on specimens obtained at autopsy I have reported that spirochetes inhabit the gastric glands and invade the parietal cells in somewhat the same way as they do in monkeys.[10] The main question is: Are human stomachs, like those of monkeys, dogs, cats and rats, regularly infected with spirochetes in the complete absence of definite related disease? If so, what is the incidence of spirochetes in human stomachs and how closely do these spirochetes resemble those in the stomach of Macacus rhesus in respect to location, associated lesions, if any, and other particulars?

An attempt has been made to secure this information by detailed study of sections of human stomachs removed at autopsy and of preparations of stomachs from monkeys.

OBSERVATIONS

From a large series, sections of 242 human stomachs were selected on the basis of good preservation and absence of marked postmortem changes, which it was thought gave opportunity for the discovery of spirochetes if they were present. The sections were stained with hematoxylin and eosin. Spirochetes were found in 103, or 43 per cent, of the specimens examined (fig. 1). It is likely that if more sections of each stomach had been studied the incidence of spirochetes would have been found to be greater. It was not possible to correlate their presence with the anatomic diagnosis. The 139 stomachs in which spirochetes were not found did not appear to differ in any significant way from the others.

Usually the spirochetes were few and were detected only after careful search. Sometimes granules were observed in the parietal cells resembling those observed by Edkins [11] in cats which had not been fed for some time. Perhaps the stomachs in question were from persons who had not taken nourishment for several hours or days before death. Eleven of the stomachs showed many spirochetes, but the infection was never so extensive as the maximum seen in the stomachs of the monkeys.

Four forms of spirochetes were seen in human gastric glands. The first two, indistinctly shown in figure 1, looked like those found in the monkey. One of these was a rather thick organism with only two or three spirals. The length varied from 4 to 8 microns, and the usual diameter was about 1 micron. This form was occasionally seen within parietal cells and was the one most frequently encountered in the human stomach. The two other forms were seen infrequently but at times were the only ones detected within a single section. Generally two of the varieties were found in the same section. The second most common spiro-

9. Macfie, J. W. S., and Carter, H. F.: Ann. Trop. Med. **11**:75, 1917.
10. Doenges, J. L.: Proc. Soc. Exper. Biol. & Med. **38**:536, 1938.
11. Edkins, J. S.: Parasitology **15**:296, 1920.

chete in the human stomach was an organism which in appearance was entirely similar to that already described but smaller in all dimensions. The length varied from 2 to 4 microns, and the average diameter was less than 0.5 micron. This spirochete also had only two or three spirals. The third form was discovered in only a few stomachs. Its length was between 8 and 20 mcirons, but it had only from three to five spirals. In diameter it was thin, being never more than 0.75 micron and usually not more than 0.5 micron. In 2 specimens spirochetes which showed sharp angulation were found. These were never abundant. This fourth form was about 6 microns in length and 0.75 micron in diameter and it had from six to eight siprals, which were equidistant and showed great regularity of shape. A similar spirochete was frequently seen in monkeys and is well shown in a parietal cell a little above and to the left of the center in figure 2 *B*.

The series of specimens from monkeys was smaller, but the tissues were removed immediately after the animals were killed and as nearly as possible from the same fundic region of the stomach. Most of them were preserved in

Fig. 1.—Spirochetes within the lumen of a human gastric gland. Note the cellular infiltration in the surrounding tissue fluid; × 2,000.

formaldehyde-Zenker solution,[12] but several were fixed in a fluid that was 9 parts absolute alcohol and 1 part solution of formaldehyde U. S. P. The sections were stained with hematoxylin and eosin. No difference depending on the fixative used was observed in the appearance of the spirochetes.

Spirochetes were noted in all of 19 monkeys given viosterol, as detailed by Cowdry and Scott,[1] and in all of 24 others which were used as controls. No consistent difference was found between the spirochetes of treated and those of untreated animals. As a rule the spirochetes were very numerous, and only in an occasional section was difficulty experienced in finding them. They occurred at all depths of the gastric mucous membrane, sometimes in the mucous lining of its internal surface and always in the necks of the glands. In the latter situation the spirochetes often appeared as deeply stained plugs of material, suggestive of extracellular multiplication (fig. 2 *A*). The row of epithelial cells on

12. This is a fixing fluid made by substituting neutral solution of formaldehyde U. S. P. for glacial acetic acid in the original formula of Zenker's solution.

EXPLANATION OF FIGURE 2

A, large masses of spirochetes in the lumen of the neck of a gastric gland of a monkey. Observe the rarefaction of the distal cytoplasm covered by the spirochetes; × 2,000.

B, spirochetes within the glandular lumen and parietal cells of a monkey. The number of spirals can be counted in two spirochetes in a parietal cell a little above and to the left of the center. In a parietal cell with a large nucleus near the upper right hand corner a spirochete is shown partly in the lumen and partly in the cytoplasm or in a canaliculus entering the cytoplasm from the lumen. Below this organism are at least 2 others within the same cell and surrounded by thin halos; × 2,000.

C, numerous spirochetes within the lumen of a monkey's gland, limited chiefly by parietal cells, the acidophilic granular cytoplasm of which is well shown. The lumen extends from the upper left to the lower right. Examining the parietal cells from above downward on the left side of the lumen, the first 2 are of normal size and appearance. The nucleus of the second even possesses a well formed nucleolus. The nuclei of the next 3 cells are by contrast smaller, blurred, irregular and somewhat fragmented. Either the spirochetes extend into the lowermost parietal cell or the cytoplasm of the cell partly surrounds the glandular lumen. The background of these spirochetes looks like the luminal background higher up in the photomicrograph; × 2,000.

D, lumen of a monkey's gastric gland. This lumen is not so straight as that in *C*. Entering the area from the right, it extends horizontally about half way across and then dips sharply downward. Above the first part of the horizontal portion is a binucleated parietal cell. In the cytoplasm to the left of the 2 nuclei is a short spirochete (or part of a long one) extending almost vertically upward and surrounded by a rather wide halo (or segment of a canaliculus). Next, on the same horizontal level, are the nuclei of 3 cells in which the cytoplasm is of small volume. These may be chief cells. Below the nucleus most distant from the right margin of the figure there is a suggestion of a spirochete. Passing now to the cells below the horizontal part of the lumen, one notes that the first 2 nuclei resemble those of chief cells, but there is a slight dip in the lumen with a definite and characteristic convergence of spirochetes into it, as if they were entering a parietal cell. The third nucleus from the right appears in section to be smaller and may belong to a cell in the tissue fluid. Extending from it to the left margin of the figure is granular cytoplasm, typical of parietal cells. The lateral cell walls cannot be distinguished, and the nuclei are faint and indistinct. The second nucleus from the left hand margin appears to have been fixed in the process of breaking up. In the lowest part of the dip of the glandular lumen, in the lower left portion of the figure, the spirochetes are oriented roughly parallel to each other and are partly within degenerating parietal cells; × 2,000.

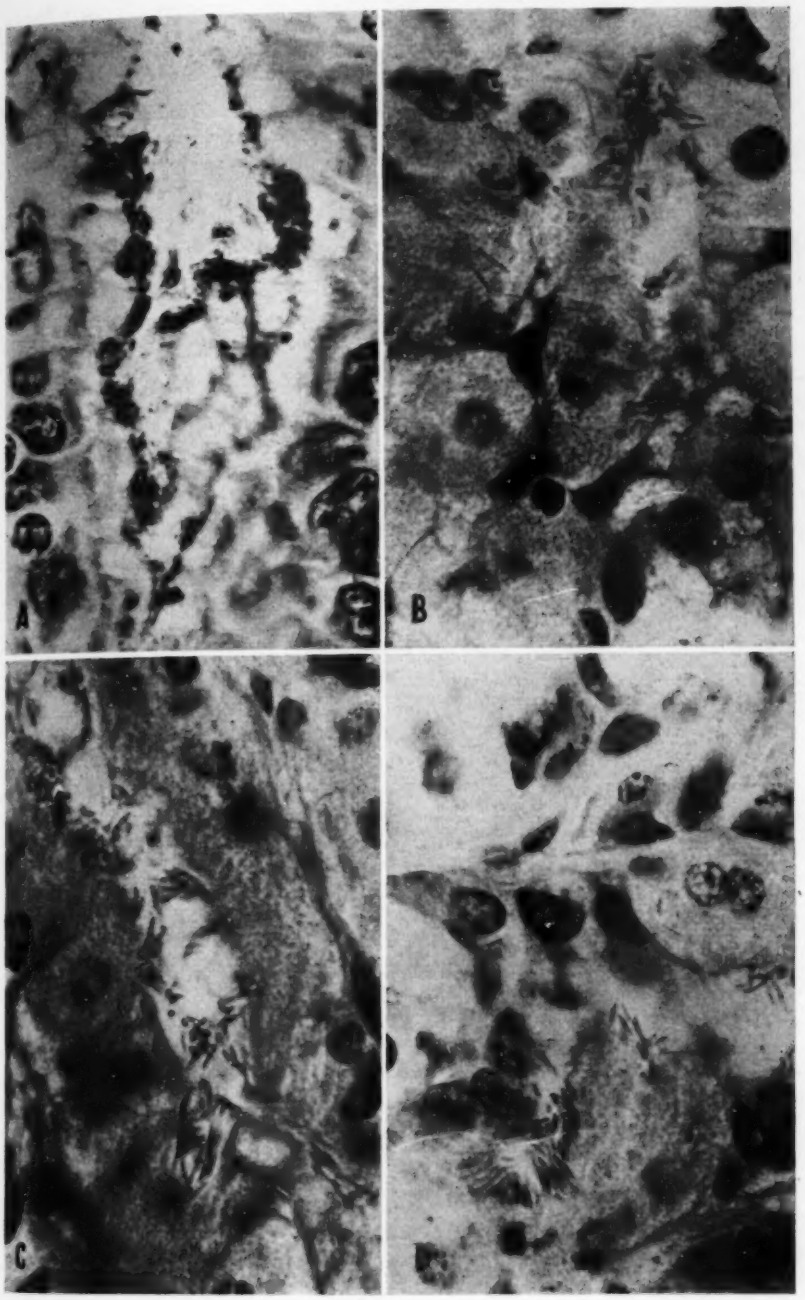

Figure 2

the left side of the lumen in figure 2*A* showed an interesting change. Their cytoplasm was divisible into two parts: an inner (proximal) and an outer (distal) part next the organisms. The latter appeared clear, as if the cytoplasm had been slightly altered by the plug of spirochetes. In other cases the cells next the spirochetes seemed to be flatter than usual. When comparatively few organisms were present in the lumens of the necks of the glands, many were found in the lumens of portions of the glands rich in body chief cells and parietal cells.

Though the majority of spirochetes were extracellular, a considerable number were intracellular, always in the parietal cells and never in goblet mucous cells or in pepsin-forming chief cells. As many as 7 were found in a single chief cell, oriented in general with their lengths parallel to its proximodistal axis. Some were seen next to the nucleus, while others were gathered about it. Signs of injury in infected chief cells were limited to the distal cell membrane and adjacent cytoplasm, when masses of spirochetes in the lumen were packed closely against it, and even then the signs were inconspicuous. The nuclei of such cells stained in the usual fashion.

In all the specimens from the monkeys spirochetes were noted within the parietal cells (fig. 2*B*), but the number in them did not appear to be in direct relation to the massive plugs of spirochetes in the lumens of the necks of the glands. Moreover, there were always more parietal cells infected than chief cells. The cell mainly involved in man, as well as in monkeys and in the other species described in the literature, is the parietal cell. In monkeys the number of organisms within a single parietal cell was generally between 2 and 8 but varied from 1 to 30. Often the entire cell was not included in a single section.

The exact position of the spirochetes within the monkey parietal cell was difficult to determine, because in hematoxylin-eosin preparations the outlines of the intracellular canaliculi were not clearly visible. It is to be remembered that Regaud[13] came to the conclusion, from the study of sections treated with the iron-hematoxylin stain, that the spirochetes of cats and dogs occur only within the canaliculi and not freely in the cytoplasm of parietal cells. The only way to settle the matter of the position in monkeys would be to stain supravitally the contents of the canaliculi by the methods of Harvey and Bensley[14]; but no living monkeys were available for the purpose. All that can be said is that in the hematoxylin-eosin specimens examined many spirochetes were present in the rather wide opening by which the canalicular system discharges into the lumen of the gland. Others were undoubtedly present within canaliculi situated more deeply in the substance of the parietal cells. Occasionally a single spirochete was observed in the cytoplasm surrounded by a faint halo (fig. 2*B*). It was not possible to determine whether the halo represented localized liquefaction of cytoplasm or a narrow canaliculus. In figure 2*B* there are two long spirochetes in a parietal cell above and a little to the left of the center. These appeared to be in close contact with the acidophilic granules. They were not throughout their lengths within recognizable canaliculi. Whether within the canaliculi or outside of them, the organisms tended to be arranged with their lengths in a proximodistal direction, but less distinctly than in the chief cells, for the parietal cells are of a more rounded shape than the chief cells. Frequently spirochetes were found apparently touching the nucleus or close to it. In general, the intracellular spirochetes occurred separately and were not closely applied together in masses of 4 or more.

13. Regaud, C.: Compt. rend. Soc. de biol. **1**:229, 1909.
14. Harvey, B. C. H., and Bensley, R. R.: Biol. Bull. **23**:225, 1912.

While all the chief cells escaped definite injury by the spirochetes except, perhaps, for slight rarefaction of the cytoplasm, which did not seem to be lethal, the parietal cells showed gradations between no apparent injury and death. The majority were not noticeably affected. In some the spirochetes were discovered in particularly close association with the nuclei, and in others they actually entered the nuclei, but this was a rare phenomenon. Some of the nuclei became shrunken, pyknotic and broken up (fig. 2 *C* and *D*). The cytoplasm became granular or vacuolated, the distal cell membrane was destroyed, and the cell substance exuded into the glandular lumen. The whole cell was sometimes cast out in this manner.

The tissue fluid just beneath (proximal to) the glandular epithelial cells was in some specimens invaded by leukocytes, mostly neutrophils and lymphocytes. A considerable number of plasma cells were seen. Though this invasion was sometimes associated topographically with cellular injury and an abundance of spirochetes, there were instances of its occurrence apart from the possibility of direct spirochetal injury, and in some glands heavily infected with spirochetes (fig. 2 *C*, to the right) the tissue fluid did not exhibit an unusual number of infiltrating cells.

It is to be emphasized that in the stomachs of both man and monkey the infection was far from uniform; one gland frequently contained many spirochetes and neighboring ones few, if any. Even in the same gland the parietal cells were affected individually, not *en masse*. Often normal-appearing cells, devoid of spirochetes, were in contact with severely injured ones.

Since all of the "infestations" occurred naturally, it was not feasible to establish a sequence of changes. There is, therefore, no evidence as to whether the spirochetes caused the injury of parietal cells or simply invaded parietal cells rather generally, some of which were near the end of their life cycle anyway and destined to disintegration and removal to make place for others.

In the monkey as in man fairly definite forms of spirochetes were observed. The first form is illustrated in figure 2 *B*, *C* and *D* but particularly in figure 2 *B*. It varied in length from 5 to 8 microns and in diameter from 0.5 to 1 micron (average 0.75). The number of sharply angular spirals varied from four to fourteen (average, six to nine). The second (fig. 2 *A*) was generally between 4 and 8 microns in length and 1 micron in diameter. It possessed of from two to four irregular and but slightly angular spirals and resembled closely the human spirochetes in figure 1. The third was a long slender organism about 0.5 micron in diameter and with only from two to four spirals in a length of 10 microns or more. It was seen only in the stomach of a single monkey. As a rule only one form was observed in a single gland. When two forms were seen in a section, there was always a great numerical predominance of one over the other.

COMMENT

The incidence of spirochetes in the gastric glands of Macacus rhesus was 100 per cent in a series of 43 animals. If the conditions for examination of human gastric glands had been more favorable, the incidence would probably have been found to be higher than 43 per cent. Bizzozero [2a] found them in all dogs which he examined. Regaud [13] considered them to be normal findings in both dogs and cats. Lim [15] looked on spirochetes in the gastric glands of cats as a more or less

15. Lim, R. K. S.: Parasitology **12**:108, 1920.

localized laboratory infection. Edkins [11] examined 105 cats from different localities and stated that it seemed permissible to regard spirochetes as just as characteristic of their gastric glands as fleas of their fur. Data for the incidence in rats [16] are less definite. In general, however, it can be said that the reported incidence is either 100 per cent or approximately so in the various species most carefully studied. It is significant also that the infection is species specific. Though present in rats, the spirochetes are absent in mice (Kasai and Kobayashi [17]), and though present in Macacus rhesus, they are apparently lacking in Cebus fatuellus (Cowdry and Scott [1]). Another point is that if they are pathogenic at all their pathogenicity is low. Kolmer and Wagner [16] looked particularly for associated lesions and stated that hyperemia and leukocytosis were absent. It is also clear that the infection centers on a particular type of cell—the parietal cell, which is concerned with the formation of hydrochloric acid. It does not ordinarily extend to the intestine. For these reasons, the conclusion appears to be justified that Macacus rhesus, and probably man also, is to be listed with the dog, the cat and the rat as a species which harbors spirochetes in the gastric glands without definite clinical symptoms and further that because of the high incidence, lack of response by the host and restricted location in cells the infection in each species is likely to be one of long standing. No evidence is available as to the age when the infection begins. Cowdry and Scott [1] found spirochetes in monkeys about 2 years old as well as in others at least 10 years old.

An important question is: Can these spirochetes, which exist in man and in the animals commonly employed for experiments and which are ordinarily harmless, be ignored in physiologic and pathologic studies on the stomach? Information on which to base an answer is unfortunately rather inconclusive and scarce. Reference has been made to Edkins' observation that in cats the spirochetes are most abundant shortly after feeding and that as the hours pass fewer spirochetes are seen even in the parietal cells, in which he found small rounded bodies disposed roughly in spiral lines suggestive of a stage in multiplication. Cowdry and Scott [1] noted no difference in spirochetal infection of Macacus rhesus after administration of viosterol over both long and short periods. Oshikawa [18] observed in gastroenterostomy of dogs that the spirochetes remained confined to mucosa containing parietal cells and did not spread to the anastomosed intestinal mucosa. Kasai and Kobayashi [17] inoculated rabbits, which are naturally devoid of gastric spirochetes, with gastric spirochetes from cats and fixed rabies virus

16. Kolmer, W., and Wagner, R. J.: Centralbl. f. Bakt. (Abt. 1) **78**:382, 1916.
17. Kasai, K., and Kobayashi, R.: J. Parasitol. **6**:1, 1917.
18. Oshikawa: Arch. f. klin. Chir. **124**:559, 1923.

and inoculated a control group with spirochetes only. In the stomachs of the former punctate hemorrhages and hemorrhagic erosions resulted. In the control rabbits no similar lesions occurred, but large numbers of spirochetes were found in the stomachs of both groups of animals.

SUMMARY

Spirochetes were found in the gastric glands of 103 (43 per cent) of 242 human stomachs examined in the routine of autopsies. They were also noted in all of 19 monkeys (Macacus rhesus) treated with viosterol and in 24 left untreated. In both the human and the monkey stomachs the spirochetes occurred in the glandular lumens and in less number in the parietal cells. Distinctive clinical symptoms were absent, and microscopic lesions associated with the spirochetes were slight or altogether lacking.

PULMONARY EPITHELIUM AND PROLIFERATIVE REACTIONS IN THE LUNGS

A STUDY OF THE CELLULAR RESPONSE IN LUNGS AFTER INTRATRACHEAL INJECTION OF TOXIC AND NONTOXIC FOREIGN SUBSTANCES

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The lining of the pulmonary alveoli and the mononuclear cells frequently seen in the alveolar spaces have been the object of numerous investigations. Recently several historical studies and reviews have been published (Miller [1]; Foot [2]; Cappell [3]; Josselyn [4]; Fried [5]) to which the reader is referred for a more extensive bibliography. In discussing the points at issue, only those considered to be current (Macklin [6]) will be treated.

LITERATURE

There are three prevalent conceptions concerning the alveolar lining. The first is that there exists a complete endodermal epithelium continuous with that which lines the other respiratory passages. Recent supporters of this point of view are Miller,[1] Foot,[2] Gardner and Smith,[7] Young,[8] Parker and Weiss,[9] El-Gazayerli,[10] Bensley and Bensley,[11] Bensley and Groff [12] and Bremer.[13] The second contends that the alve-

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1. Miller, W. S.: The Lung, Springfield, Ill., Charles C. Thomas, Publisher, 1937; in Cowdry, E. V.: Special Cytology, New York, Paul B. Hoeber, Inc., 1928, p. 71.
2. Foot, N. C.: Am. J. Path. **3**:413, 1927.
3. Cappell, D. F.: J. Path. & Bact. **32**:625, 1929.
4. Josselyn, L. E.: Anat. Rec. **62**:147, 1935.
5. Fried, B. M.: Arch. Path. **17**:76, 1934.
6. Macklin, C. C.: J. Thoracic Surg. **6**:82, 1936.
7. Gardner, L. U., and Smith, D. T.: Am. J. Path. **3**:445, 1927.
8. Young, J. S.: (*a*) J. Path. & Bact. **31**:265 and (*b*) 705, 1928; (*c*) **33**:363, 1930.
9. Parker, F., Jr., and Weiss, S.: Am. J. Path. **12**:573, 1936.
10. El-Gazayerli, M.: J. Path. & Bact. **43**: 537, 1936.
11. Bensley, R. D., and Bensley, S. H.: Anat. Rec. **64**:41, 1935.
12. Bensley, S. H., and Groff, M. B.: Anat. Rec. **64**:27, 1935.
13. Bremer, J. L.: Contrib. Embryol. (no. 83) **25**:83, 1935.

olar wall is incompletely covered by surviving endodermal cells. This has been maintained of late by Palmer,[14] Wenslaw [15] and Brodersen.[16] The third holds that the wall is completely divested of its original epithelium and is composed only of cells of mesodermal origin (Josselyn [4]; Loosli [17]; Fried [18]).

As for the alveolar phagocyte (dust cell, *Hertzfehlenzell,* and so on), current authorities on this may be divided arbitrarily into three schools. First are those investigators who believe that this cell may arise from the pulmonary epithelium. Among them Cappell [3] and Carleton [19] have done recent work. Second is the school which holds it to be of mesodermal origin (Miller [1]; Foot [2]; Gardner and Smith [7]; El-Gazayerli [10]; Josselyn [4]; Loosli [17]; Fried [18]; Bloom [20]; Wright [21]; Ungar and Wilson [22]). The third school may be disregarded. To my knowledge, no one who in the last ten years has worked primarily to investigate this cell now believes the alveolar phagocyte to be derived from endothelium.

The different methods which have been applied to the study of these problems are reviewed here and criticized briefly in order to introduce the rationale which lies behind the present work.

In general, the methods employed may be listed as follows: (1) The outlining of the epithelial cells by specific staining; (2) the employment of vital and supravital technic; (3) embryologic and phylogenetic studies; (4) the treatment of basement membranes with stains for connective tissue to differentiate cell layers; (5) the alteration of the architecture of normal lungs in an endeavor to make epithelium more visible, and (6) the production of proliferation of the constituent cells.

In the first group, Bensley and Bensley [11] used intravenous gold sodium thiosulfate to demonstrate ground substance, silver stains by intratracheal injection to outline epithelial cells and Bielschowsky stains for reticular fibers. They concluded that the pulmonary alveoli of adult mammals are lined by a continuous cellular membrane. On the other hand, Josselyn [4] applied similar methods and came to the conclusion that

14. Palmer, D. M.: Am. J. Anat. **58**:59, 1936.

15. Wenslaw, A.: Compt. rend. Soc. de biol. **104**:611, 1930.

16. Brodersen, J.: Ztschr. f. mikr.-anat. Forsch. **32**:73, 1933.

17. Loosli, C. G.: Anat. Rec. **62**:381, 1935.

18. (*a*) Fried, B. M.: Arch. Path. **18**:865, 1934. (*b*) Fried, B. M., and Whitaker, L. R.: Arch. Int. Med. **40**:726, 1927. (*c*) Fried, B. M.: Arch. Path. **3**:751, 1927; footnote 5.

19. Carleton, H. M.: Proc. Roy. Soc., London, s.B **114**:513, 1934.

20. Bloom, W.: Arch. Path. **3**:608, 1927.

21. Wright, R. D.: Am. J. Path. **11**:497, 1935.

22. Ungar, J., Jr., and Wilson, G. R.: Am. J. Path. **11**:681, 1935.

the black lines seen on alveolar septums in silver nitrate impregnations of lungs may not be the boundaries of living cells. Moreover, he maintained that the alveolar wall is lined by a homogeneous ground substance on which the continuity of the cellular membrane depends.

Among the embryologists, Bensley and Groff [12] worked with macerated lungs of young rats at various stages from 20 day embryos to adults. They described the cuboidal alveolar epithelium as persisting until the twentieth day of gestation. After that time it became stretched into a thin membrane with successive inspirations just before and after birth, remaining thereafter as a distinct membrane demonstrable in adult life. Wenslaw [15] studied late stages of embryonic life in man. He, in turn, claimed that in some places the pulmonary epithelium acquires an apparent discontinuity at the end of fetal life but that under certain conditions it reverts to the primitive condition of a continuous layer of cells loaded with lipoid and lining the alveoli.

Gardner and Smith [7] studied normal guinea pig lungs supravitally stained with neutral red. They concluded that the alveolar epithelium and the local vascular endothelium play no part in the origin of the alveolar phagocyte. On the other hand, Cappell [3] maintained that those cells reacting to particulate dyes introduced intratracheally are derived from cells in the alveolar lining and confine their activities to those spaces bounded by pulmonary epithelium.

By a combination of the fourth and fifth technics, Loosli [17] studied lungs after collapse by phrenicotomy and pneumothorax and ligation of the pulmonary veins. He used the Mallory-Azan technic to stain ground substance and contended that: "At present there is no morphologic or histologic method which can demonstrate that the nucleated cells in the capillary meshes in the alveolar septa form a continuous histologic epithelium lining the spaces in the respiratory portion of the collapsed or expanded rabbit's lungs."

Parker and Weiss [9] studied lungs altered by the changes occurring in mitral stenosis, using the Lee-Brown modification of Mallory's stain for connective tissue. In this manner they combined the fourth with the sixth approach and concluded that the alveolar wall is covered by flattened epithelial cells.

The approach which aims to induce cellular proliferation has, in turn, resulted in conclusions at variance with one another. Miller [1] studied desquamation as it occurs in lobar pneumonia. Foot [2] worked with supravitally stained films and paraffin sections of supravitally stained lungs in which milk pneumonia had been induced. Young [8] used irritants introduced intrapleurally. El-Gazayerli [10] added cytologic specific staining to Young's approach and described del Rio Hortega's silver carbonate stain as being specific for phagocytes. Sprunt, Martin

and Williams,[23] following up work done by Winternitz and his associates [24] on reactions in the lungs in which alveolar epithelial proliferation plays an important role, used bacteria and bacterial toxins. All these investigators were convinced of the integrity of the alveolar epithelial lining. On the other hand, Fried [18] worked with vital stains and foreign bodies of a nontoxic nature introduced intratracheally. He expressed the opinion that there is no endodermal epithelial lining to the alveolus but that the large mononuclear cells found in the pulmonary alveoli in inflammatory and congestive processes are macrophages, originating from cells of mesenchymal origin found along the walls of the air vesicles.

It seems that much of the confusion in the past has been due to the fact that until very recently little attention has been paid to a correlation between the type of reaction and the strength of stimulus used, or to the time element concerned in the various inflammatory reactions in the lungs. Thus Fried,[18b] using a nontoxic foreign substance (iodized poppyseed oil 40 per cent), brought out macrophages [25] with very little epithelial proliferation. Again, Cappell [3] killed his animals only one-half hour after injecting one dye intravenously and another intratracheally. The intratracheal dye remained in cells on one side of the alveolar wall and the intravenous dye was seen only in cells on the other side. Thereupon he concluded that the alveolar phagocytes arise from the cells of the alveolar lining and do not enter the underlying tissues.

Closer attention has recently been given to the time factor in studies of inflammatory processes in the lungs. Young [8c] found that the visible cycle of changes in the pulmonary alveolar epithelium of the rabbit covers a period of eight days after the initial stimulus has been administered, waxing to cellular proliferation on the third or fourth day and thereafter regressing. Moreover, Sprunt, Martin and Williams [23] noted that on the third or fourth day after intratracheal administration of staphylococcus toxin, when the polymorphonuclear cell reaction had begun to subside, the walls of the alveoli and the tissues surrounding the bronchi and bronchioles showed an infiltrate of lymphocytes and monocytes. They also pointed out that, in addition to the presence of these cells, the walls of the alveoli became definitely thickened with cuboidal cells which had vesicular nuclei and scanty, pale-staining cytoplasm. These cells were believed to be epithelial, but it was not possible

23. Sprunt, D. H.; Martin, D. S., and Williams, J. E.: J. Exper. Med. **62:** 73 and 449, 1935.

24. Winternitz, M. C.; Smith, G. H., and McNamara, F. P.: J. Exper. Med. **32:**199, 205 and 211, 1920.

25. In the following pages all cells of the mononuclear phagocytic series will be referred to as macrophages.

to determine their exact nature because of limitations imposed by the hematoxylin and eosin–stained material.

The present study was undertaken to see whether vital and supravital staining of the lungs in such a series of animals would aid in distinguishing between these two types of cells. Furthermore, it was thought that a comparative study of the reactions in the lungs to a substance of toxic nature and to one of a nontoxic nature might be of value. Each of the substances used to induce cellular proliferation had led its respective employer in the past to contradictory conclusions on the nature of the alveolar wall. To determine, if possible, what it was that caused this divergence of opinion and thereby to throw further light on the nature of the cells in question, the following experiments were undertaken.

METHODS AND MATERIALS

Twenty-one young adult rabbits, weighing between 2 and 4 Kg., were used in this experiment. The presence of Bacterium leptisepticum and Bacillus bronchisepticus in the nares was ruled out by means of cultures after silver nitrate instillations, as suggested by Meyer.[26]

Toxin.—Toxin was made from the hemolytic strain of Staphylococcus aureus employed by Rigdon and his associates[27] and was prepared by the method described by Parker, Hopkins and Gunther.[28] The content of one vaccine bottle of toxin was employed throughout all these experiments.

Iodized Oil.—The iodized oil used was poppyseed oil 40 per cent (lipiodol-Lafay N.N.R.).

Inoculation.—The technic described by Sprunt, Martin and Williams[23] was employed for intratracheal inoculation.

Dose.—Preliminary experiments showed that 0.05 cc. of the toxin per animal produced the optimal proliferative type of lesion, while large doses produced a hemorrhagic and necrotizing lesion and in some cases, death within two or three days. The toxin was always made up to 1 cc. with physiologic solution of sodium chloride.

Iodized oil was administered in doses of 1 cc. per kilogram, an amount which was considered optimal by Fried[18b] in working with cats.

Necropsy.—With the exception of 2 rabbits stained by intravascular perfusion, each animal was killed by a blow at the base of the skull, and necropsy was performed at once. The gross changes were not significant, so only the microscopic ones are given.

Fixation and Staining.—The lungs of 2 animals were stained with neutral red according to Hu's[29] modification of Forkner's[30] technic. Those of 16 other

26. Meyer, K. F., in Jordan, E. O., and Falk, L. S.: The Newer Knowledge of Bacteriology and Immunology, Chicago, University of Chicago Press, 1928, p. 607.

27. Rigdon, R. H.; Joyner, A. L., and Ricketts, E. T.: Am. J. Path. **10**: 425, 1934.

28. Parker, J. T.; Hopkins, J. G., and Gunther, A.: Proc. Soc. Exper. Biol. & Med. **23**:344, 1926.

29. Hu, C. H.: Proc. Soc. Exper. Biol. & Med. **29**:258, 1931.

30. Forkner, C. E.: J. Exper. Med. **52**:379, 1930.

animals were removed immediately after death and immersed in a beaker of Forkner's staining solution. This was maintained at 37 C. in a water bath, and the lungs were gently perfused for fifteen minutes with the same solution introduced through the trachea. The inflow of staining solution was regulated so as not to overdistend the lungs and was followed by fixation after the method described by Hu.[29] Dehydration and clearing were carried out as described by Hu [29] in all cases except one. In this case the technic described by Gardner [31] was used.

In the case of the lungs stained with neutral red, three sections from each block were prepared for study. One of these was stained with hematoxylin and eosin. This usually resulted in washing out all the neutral red. The second was stained for nuclear detail by the technic described by Gardner,[31] using Harris' hematoxylin without acetic acid. The third was studied stained only with neutral red after dissolving excess mercuric chloride crystals in aqueous solution of iodine. All observations with low magnification were best made after staining with hematoxylin and eosin. The sections stained using Gardner's technic allowed comparisons of nuclear structure to be made between sections stained with neutral red alone and those stained with hematoxylin and eosin. The plain neutral red sections made possible a better discrimination between cells which were alive and cells which were dead at the time the animal was killed.

The lungs of animals which received carmine and trypan blue were fixed by gentle intratracheal perfusion with 10 per cent neutral solution of formaldehyde U. S. P. Sections were stained with brazilin for study under high magnification, and others with hematoxylin and eosin.

EXPERIMENTAL OBSERVATIONS

Experiment 1.—This experiment was designed to allow a study of changes in the lungs after administration of optimal doses of toxic and nontoxic substances.

Fourteen rabbits were used. Six received 0.05 cc. each of staphylococcus toxin intratracheally. These were killed after thirty-two, forty-eight, seventy-two, ninety-six hours and eight days, respectively. The ninety-six hour experiment was represented by 2 animals. The other 8 animals received 1 cc. each of iodized oil per kilogram and were killed at the same intervals, the ninety-six hour experiment being represented by 4 animals. The lungs of these animals were stained with neutral red and sections prepared for study as described in the foregoing section.

Changes Produced with 0.05 Cc. of Toxin.—After thirty-two hours the reaction to toxin was characterized by rather copious exudation of cells into the terminal air spaces about the larger bronchi, as shown in figure 1, *1*. The majority of these cells were active macrophages. A few degenerating macrophages and polymorphonuclear cells were present, but both were being phagocytosed. Small amounts of serum and fibrin were seen in the alveoli. Slight thickening of the alveolar septums was observed. This was occasioned by moderate edematous swelling and by increase of cells within the septums. The bronchial epithelium appeared to be intact.

As in the lungs undisturbed by pathologic change, the alveolar walls for the most part seemed to have no distinct cellular lining. In many places the capillaries seemed to be exposed to the alveolar air. In others, flattened cells with darkly stained oval nuclei seemed to have separated from the surface of the alveoli.

31. Gardner, L. U.: Proc. Soc. Exper. Biol. & Med. **24**:646, 1927.

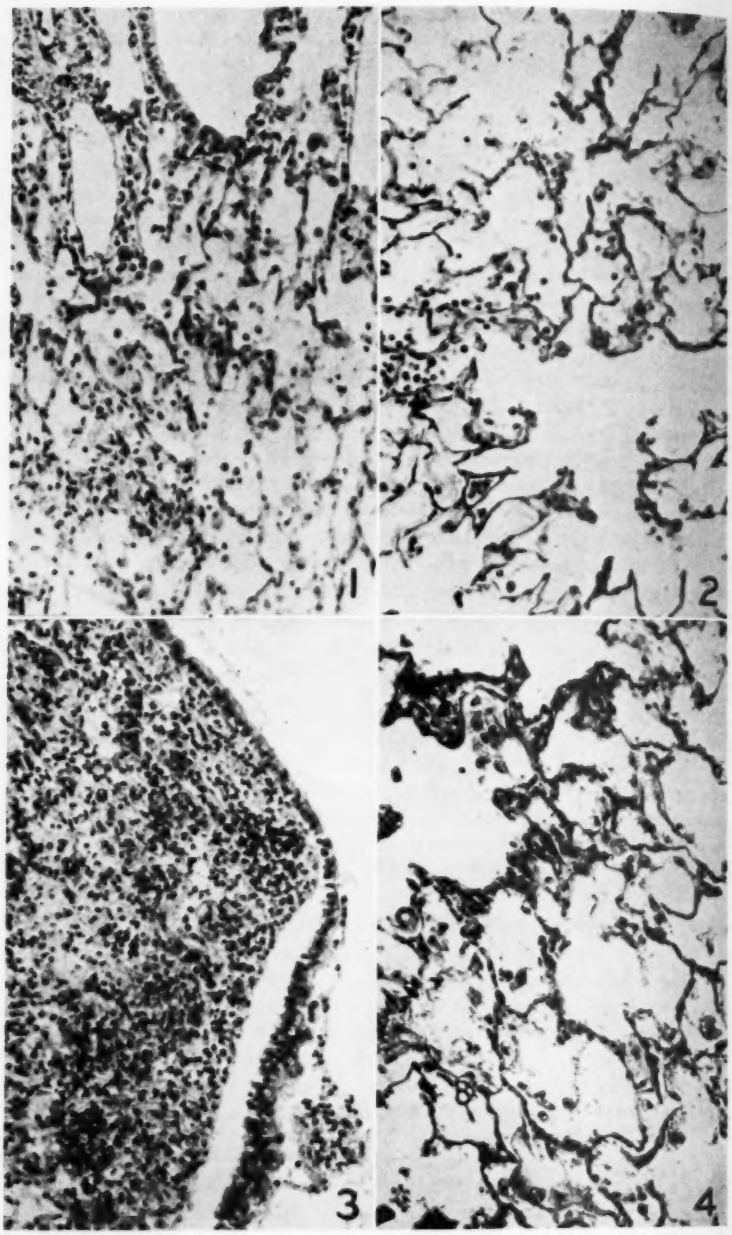

Fig. 1.—*1*, thirty-six hours after intratracheal injection of 0.05 cc. staphylococcus toxin. *2*, thirty-six hours after intratracheal injection of 1 cc. per kilogram of iodized oil. *3*, forty-eight hours after intratracheal injection of 0.05 cc. of staphylococcus toxin. *4*, forty-eight hours after intratracheal injection of 1 cc. per kilogram of iodized oil. Hematoxylin and eosin; × 85.

In a few places a group of fibroblasts projected into the alveolar space. Rarely such a group was seen to project on both sides of one septum so that their connecting protoplasmic bands apparently passed through the septal wall.

After two days areas of heavy consolidation about the bronchi were seen, and the alveoli in these areas were filled with a serous exudate containing great numbers of leukocytes. When this reaction was most severe, exudation of leukocytes and of red cells was so great as to obscure the pattern of the lung. The white cells of the exudate were chiefly degenerated macrophages and polymorphonuclear cells, although numerous large active macrophages were present. The bronchial epithelium showed considerable degenerative change and in places was desquamated in sheets.

The edematous swelling, cellular infiltration and vascular engorgement of the walls of the alveoli, coupled with the intense serous and cellular exudate into the alveoli themselves, made definition of a lining membrane impossible within the areas of intense inflammatory reaction at this stage.

After three days the microscopic appearance (fig. 2, 5) was still one of rather heavy consolidation about the large bronchi. Evidences of destruction and of proliferation were seen in the bronchial epithelium, and in places there were masses of red and white cells caught in strands of fibrin within the bronchi. In patches within areas of consolidation, retraction of the exudate from the alveolar walls permitted the architectural pattern of the lungs to be seen. Once again the large, active macrophages had come into prominence, although they were still outnumbered by degenerated cells. Numerous giant cells were present. Only in those places where no retraction had occurred were there great numbers of red cells and polymorphonuclear cells within the alveoli. An explanation of the rapid removal of these degenerating cells, along with the remainder of the previous day's alveolar exudate, could be seen in the longitudinal section of the termination of a bronchiole. This could be followed for a considerable distance, starting at its ramifications among alveoli, which showed some evidence of resolution. Near its terminal branches the predominant cells were active large macrophages. As observation proceeded proximally, the character of the exudate changed and became more nearly like that seen in the alveoli on the previous day. The nuclei of the macrophages became pyknotic, the polymorphonuclear cells were largely disintegrated, and the red blood cells were represented, for the most part, by a homogeneous matrix of orange pigment.

In those areas in which the exudate had separated from the septum, a change in the alveolar lining was evident. In these places there was a definite cellular border on the inner surfaces of the alveoli. The constituents of this border were very low cuboid cells, generally with round reticular nuclei which had one or two nucleoli and a rather heavy nuclear membrane. Occasionally the nuclei were somewhat oval, with their long diameter parallel to the surface of the alveolar wall. These cells were seen to be contiguous, the distance between adjacent nuclei being usually about one third of the diameter of the nucleus itself. It was not difficult to trace this cellular alveolar lining from the alveoli and terminal ramifications of the air ways to the walls of the bronchioles, where it gradually assumed a more compact cuboidal arrangement; then, to the columnar epithelium of the bronchioles and bronchi. It was very difficult to distinguish the cells lining the alveoli from the large macrophages of the exudate on the basis of cytologic characteristics alone when stained with hematoxylin and eosin. In regions of epithelial proliferation the septums were very greatly thickened, the capillaries within them were dilated and the proliferation of fibroblasts between the rows of cuboid cells was still evident.

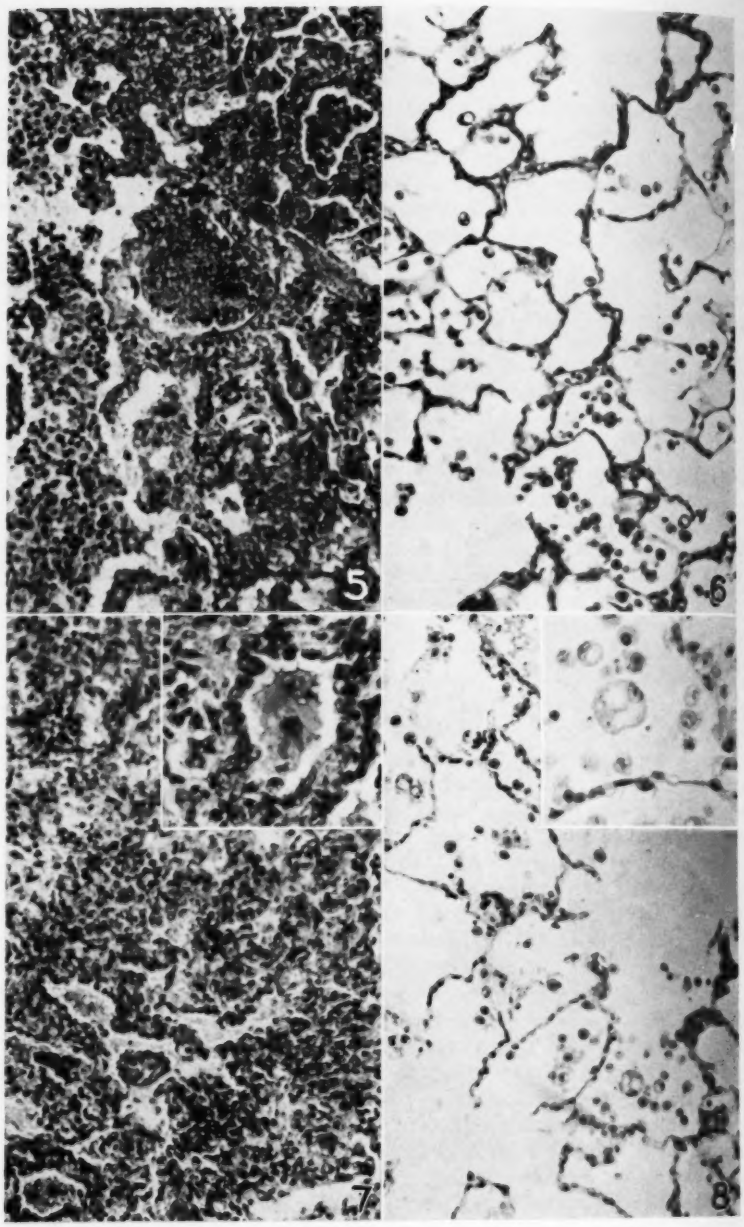

Fig. 2.—*5*, seventy-two hours after intratracheal injection of 0.05 cc. of staphylococcus toxin. *6*, seventy-two hours after intratracheal injection of 1 cc. per kilogram of iodized oil. *7*, ninety-six hours after intratracheal injection of 0.05 cc. staphylococcus toxin; inset shows proliferated alveolar epithelium. *8*, ninety-six hours after intratracheal injection of 1 cc. per kilogram of iodized oil; inset shows macrophages with clear cytoplasm. Hematoxylin and eosin. The insets in *7* and *8* show magnification × 173.3; the parts otherwise show magnification × 86.7.

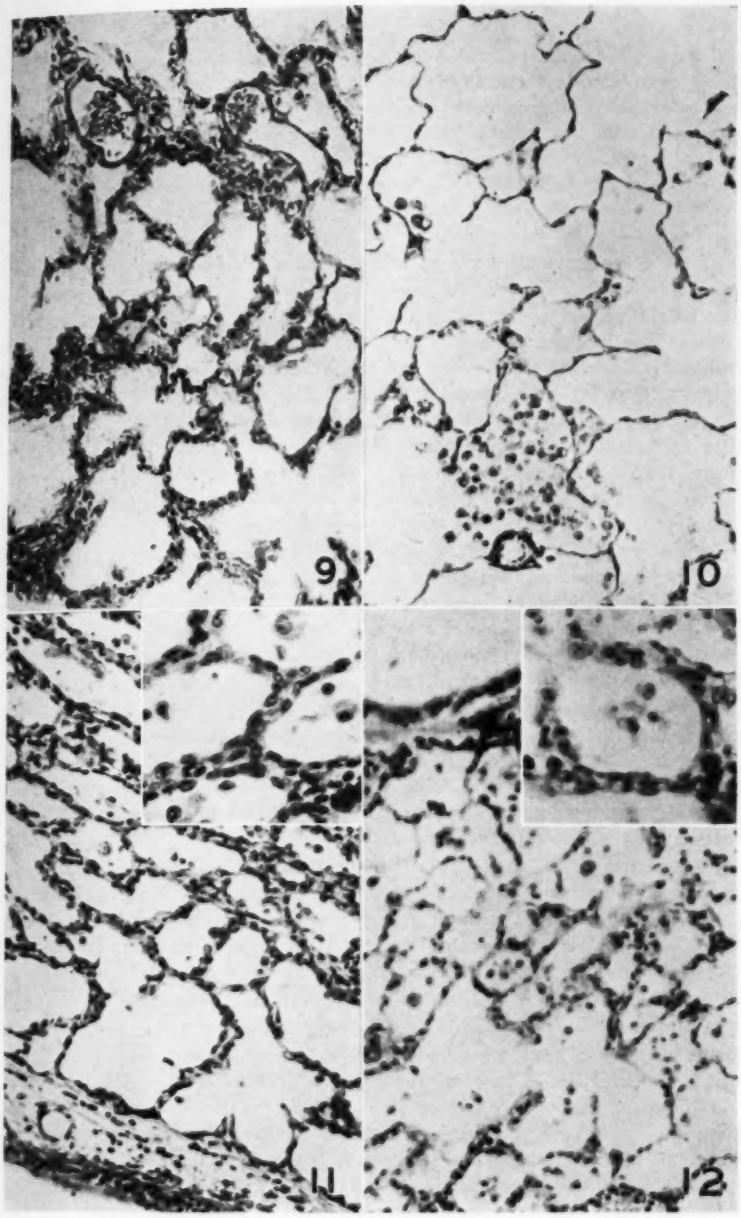

Fig. 3.—*9,* eight days after the intratracheal injection of 0.05 cc. of staphylococcus toxin. *10,* eight days after the intratracheal injection of 1 cc. per kilogram of iodized oil. *11,* ninety-six hours hours after intratracheal injection of 0.03 cc. of staphylococcus toxin; inset shows proliferated epithelium. *12,* ninety-six hours after the intratracheal injection of 0.01 cc. of staphylococcus toxin. Compare inset with insets *7* and *11.* Hematoxylin and eosin. The insets show magnification × 180; otherwise the parts show magnification × 86.7.

After four days the general microscopic appearance (fig. 2,*7*) was still one of dense consolidation about the larger bronchi. Desquamation and proliferation of the bronchial epithelium were still evident. The exudate within the bronchi as compared with that of the previous day seemed to be chiefly of a serous or mucinous nature. The few cells seen within it were no longer a conglomeration of degenerated red and white cells but fairly large macrophages and giant cells (in fig. 4, *16* and *17*) such as were seen in those alveoli of the previous day which had begun to show evidences of resolution. The vast majority of the cells of the alveolar exudate were not active macrophages. The alveolar septums were still greatly swollen. However, the most striking and characteristic feature of the picture presented by the lungs at this stage was the distinct layer of cuboid cells lining the alveoli in places where the process of resolution was in progress. Compared with those of the previous day, more alveoli displayed this lining, and its cells were more definitely cuboid. It was evident that in places that exudate had undergone fibrous organization and had, in turn, become epithelialized. In consequence, some of the terminal air spaces showed both the septal wall and the exudate within covered by an epithelial pavement. Continuity from bronchial to alveolar epithelium was demonstrable here, although the sections were not cut as fortunately as were those of the previous day.

In the neutral red sections the cells of the exudate were seen to be typically stained macrophages, the cytoplasm of which was loaded with brilliant orange and brick red vacuoles of various sizes. The smaller cells seemed to contain the finer vacuoles whereas in the larger some of the vacuoles attained a size nearly equal to half the diameter of the nucleus. Around alveoli which contained 5 to 10 such well stained macrophages the cuboid epithelial cells were seen to contain no dye or only a few specks of it (fig. 4,*15*). This contrast was clear and distinct. The neutral red was given this animal by intravascular perfusion, so that the stain must have passed through the epithelial border to gain access to the phagocytes inside the alveoli. It is to be noted that where the staining was intense the tall columnar cells of the bronchial epithelium contained very fine dark droplets of neutral red.

By the eighth day a large amount of resolution had occurred (fig. 3,*9*). The exudate had almost entirely disappeared from the alveoli. No longer was there any evidence of epithelial proliferation. The only record of the preceding disturbance was that manifest in the interstitial tissues of the septums. These were still considerably thickened by an abundance of what proved to be collagenous tissue when stains for connective tissue were applied.

Changes Produced with Iodized Oil.—After thirty-two hours there were large numbers of macrophages with vacuolated cytoplasm within alveoli and smaller bronchioles (fig. 1,*2*). These macrophages had taken up neutral red in vacuoles of various sizes. However, it seemed that the more large clear vacuoles a cell contained, i. e., the more iodized oil it must have ingested, the fewer were the vacuoles of neutral red. In a few places the alveolar septums were slightly thickened by very mild proliferation of fibroblasts.

The flat cells with oval nuclei thought to line the alveoli had taken up no neutral red. Macrophages were frequently seen projecting from the alveolar walls, but these did not resemble an epithelium.

After forty-eight hours there was little change in the microscopic picture (fig. 1,*4*) beyond that described in the foregoing paragraph. There was a slight increase in the number of vacuolated macrophages in the exudate, and once again there seemed to be a definite connection between the amount of iodized oil and the amount of neutral red phagocytosed by the cells. There was a slight

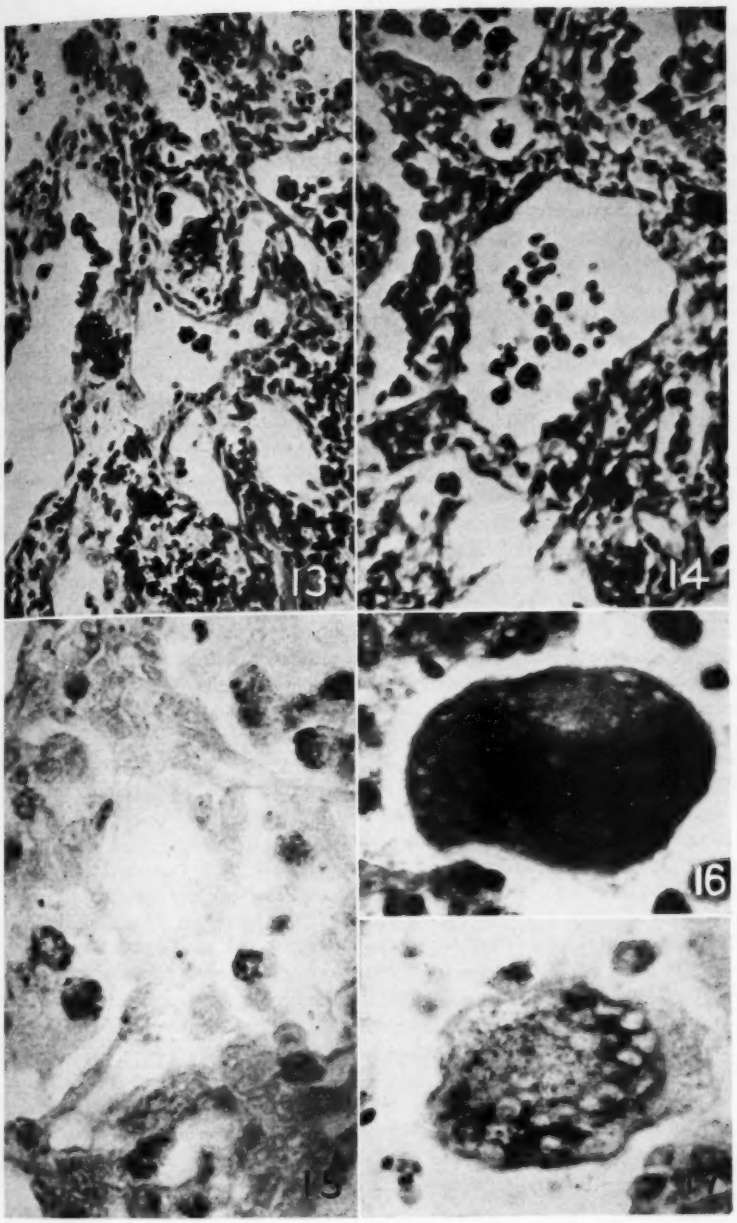

Fig. 4.—*13*, seventy-two hours after intratracheal injection of 0.05 cc. of staphylococcus toxin (carmine injected intravenously). Brazilian counterstain; × 173.3. *14*, same as *13*. *15*, ninety-six hours after intratracheal injection of 0.05 cc. of staphylococcus toxin. Neutral red and hematoxylin; × 390. *16*, ninety-six hours after intratracheal injection of 0.05 cc of staphylococcus toxin. Hematoxylin and eosin; × 390. *17*, same as *16*, stained with neutral red and hematoxylin; × 390.

increase in the septal thickening produced by interstitial proliferation of fibroblasts. There was a slight serous exudate in some of the alveoli.

In a very few places where cells lining the alveoli appeared to be present in continuous flattened sheets, there was indication of epithelial proliferation. These cells contained no neutral red. Macrophages in small aggregations were seen in the loose connective tissues.

At the end of seventy-two hours the general microscopic appearance had been altered very little (fig. 2, *6*). There seemed to be a few more cells in the alveolar exudate, including a few polymorphonuclear cells. Practically none of the serous exudate was left within the alveoli. The changes in the septums were about the same as they were on the previous day. Where epithelial proliferation had occurred, it was very slight. The bronchial epithelium remained intact.

The cells of the exudate, although still practically all macrophages, had changed somewhat in appearance. The large clear vacuoles had in general become smaller and were more numerous within the individual cells. The reciprocal relationship between the number of clear cytoplasmic vacuoles and those filled with neutral red was again evident. Moreover, there was a new order of smaller macrophages which contained no clear cytoplasmic vacuoles but which had taken up neutral red in small dark vacuoles arranged in a rosette at the hof of the nucleus. Macrophages in the loose connective tissues were more in evidence than on the previous day.

There was little difference between the picture presented at ninety-six hours (fig. 2, *8*) and those of the previous three days. Rare instances of alveolar epithelial proliferation were seen in which cuboid cells were arranged in continuous sheets along the alveolar walls. These cells contained no vacuoles of neutral red. Comparatively few of the macrophages in the alveoli contained large clear cytoplasmic vacuoles. More of them contained fairly numerous neutral red vacuoles of varying sizes and shapes. The polymorphonuclear cells gave evidence of degeneration and many of them had been ingested by macrophages.

After eight days the reaction had subsided to a marked degree, as shown in figure 3, *10*. A great majority of the alveoli were entirely clear, although occasional air spaces were seen to contain rather large aggregations of macrophages and débris. In a few of these, epithelial proliferation was evident. More alveoli contained 1 or 2 free macrophages than is the case in sections of normal lungs, but only a few of these scattered macrophages showed vacuoles containing iodized oil, whereas the majority in the large aggregations did possess them. Most of the macrophages gave evidence of having undergone some degree of degeneration. Their cytoplasm appeared frayed and their nuclei pyknotic. Most of them contained neutral red in only a few, fairly large vacuoles. They seemed to be older cells which had lost a large measure of their phagocytic power.

Experiment 2.—This experiment was designed to show whether the differences in result between toxin and iodized oil were due to a qualitative difference in the toxin or merely to a quantitative difference in the toxicity of the two substances.

Of the 4 rabbits used in this experiment 2 were given 0.03 cc. each of toxin and 2 received 0.01 cc. each of toxin. All the animals were killed four days after the injections.

Changes Produced with 0.03 Cc. and with 0.01 Cc. of Toxin.—Four days after the administration of 0.03 cc. of toxin the reaction (fig. 3, *11*) was by no means as intense as that seen after 0.05 cc. had been used. The bronchial epithelium was intact, and the alveoli were not choked with the copious exudate seen after

the larger dose. The most striking similarity was in the epithelial proliferation. Because the exudate was generally so slight, the epithelial cells stood out very clearly. Everywhere within the areas of reaction, on both sides of the alveolar septums sheets of low cuboid cells with slightly oval nuclei were seen. The epithelial border thus produced was seen to be continuous all the way around the alveoli, and where the sections had been cut opportunely, it was possible to trace the continuity of epithelium from the bronchi and bronchioles through what must have been the alveolar ductules and the alveolar atria and saccules into the alveoli of the lungs. These cells remained for the most part unstained by neutral red. At times very fine specks of the dye were seen near the nuclei. The macrophages of the exudate in contrast to those seen after administration of the larger dose of toxin had not taken up the stain with great avidity. Their cytoplasmic vacuoles, although of fair size, were comparatively few. A great many of these cells contained no vacuoles at all, but their nuclei had taken up the stain. Again the septums were thickened by proliferation of fibroblasts, among which were seen a few macrophages.

It would be a very difficult matter to distinguish the reaction to 0.01 cc. of this staphylococcus toxin (fig. 3, *12*) from that incited by the administration of 1 cc. of iodized oil per kilogram. Practically the only distinguishing feature was that the macrophages in the exudate did not contain the large clear cytoplasmic vacuoles. The bronchial epithelium appeared to be intact. There was no consolidation, and the cells in the exudate were practically all large macrophages. They appeared to be undergoing varying degrees of cytolysis and contained even less neutral red than in the preceding case. In many places they were seen to have been ingested by cells of their own species. The few giant cells present contained only 2 or 3 nuclei. Where septal thickening existed, it was occasioned by moderate proliferation of fibroblasts. Only in areas where the reaction was strongest had there occurred slight epithelial proliferation.

Experiment 3.—The third experiment was undertaken for only one purpose. This was to investigate whether or not macrophages of the exudate were capable of wandering from the connective tissue into the alveoli and back again, for it might be contended that they were only specialized epithelial cells.

Three rabbits were used. Each received 1 cc. of 1 per cent trypan blue intravenously for three days before receiving 0.05 cc. of toxin intratracheally. Forty minutes after the toxin was introduced 3 cc. of 1 per cent carmine was put into the trachea of one of these animals. The forty minute delay was interpolated to forestall adsorption of the toxin by carmine. On the day of inoculation another animal received 1 cc. of 1 per cent carmine intravenously and 1 cc. by the same route on each of two successive days before death. The third animal continued to receive daily intravenous injections of 1 cc. of 1 per cent trypan blue. All 3 were killed at seventy-two hours.

Distribution of Vital Dyes.—The reaction was similar to that found in the rabbits which were killed three days after receiving 0.05 cc. of the toxin. The changes are shown in figure 4, *13* and *14*. Macrophages containing carmine or trypan blue or both were seen in the alveoli and also in the interstitial tissue.

SUMMARY OF ANATOMIC CHANGES

In brief, the reactions in the lungs which formed the basis of this study took place as follows: First there was moderate exudation of polymorphonuclear cells. This was rapidly followed by outpouring of

large numbers of macrophages, which ingested the polymorphonuclear cells. Within the first thirty-two hours there occurred moderate proliferation of fibroblasts within the septal walls, and degenerative changes became evident in the pulmonary epithelium. These changes were made manifest by a mild amount of epithelial desquamation. Where this occurred, fibroblasts were sometimes seen to grow from septal interstices into the alveoli, and when they projected from both sides of one septum the appearance was that of alveolar pores (Miller;[18] Macklin;[32] Loosli [33]). During the second day the epithelial damage became further evident. Serum and red cells seeped out into the alveoli, and there was further outpouring of macrophages and polymorphonuclear cells. By this time the proliferation of macrophages within the connective tissues of the lungs themselves became strikingly evident. Multinucleated giant cells were plentiful in the exudate. The bronchial epithelium began to show degenerative changes, and in many places it was desquamated in sheets. By the third day the reaction began to resolve. A large amount of exudate was apparently ejected via the larger respiratory passages. The macrophages attained their greatest numbers and were seen to ingest residual débris. Proliferation of alveolar epithelium became evident along the septal borders, and in places epithelialization of the exudate occurred. By the fourth day this epithelial proliferation reached its height. Sections of vitally stained tissue showed these cells to be nonphagocytic and distinct from the macrophages present. Moreover, their continuity with the cells lining the bronchioles and bronchi could be traced. Quite a few small round cells, judged to be degenerated macrophages, were seen in the exudate. After eight days the only evidence of the damage sustained by the tissues was a moderate thickening of septal walls occasioned by proliferation of fibroblasts. Even these appeared to be shrunken, as judged by their nuclei and by the amount of collagenous tissue present.

After the introduction of 1 cc. of iodized oil per kilogram into the trachea, no such severe inflammatory reaction occurred. It was obvious that little, if any, damage was sustained by the pulmonary epithelium. If an initial polymorphonuclear cell reaction occurred, it was missed in this study. By the end of the first thirty-two hours large numbers of macrophages were seen within the alveoli. They seemed to have taken up large quantities of the iodized oil, as evidenced by their colorless cytoplasmic vacuoles. By the end of the third day many of the original macrophages seemed to have undergone degenerative changes. At this time many younger macrophages and a few polymorphonuclear cells were present in the exudate. The former were seen to ingest cells of their

32. Macklin, C. C.: J. Anat. **69**:188, 1935; Arch. Path. **21**:202, 1936.
33. Loosli, C. G.: Arch. Path. **24**:743, 1937.

own species, as well as degenerated polymorphonuclear cells. Small aggregations of macrophages within the connective tissues about blood vessels and bronchi of the lungs were seen. The only other interstitial reaction was a very slight proliferation of fibroblasts within the walls of a few of the septums. Very rarely did epithelial proliferation occur. By the eighth day the majority of the remaining macrophages seemed to be aggregated in isolated air spaces.

That the difference between these two reactions was one of degree and not specific was illustrated by the titration series. This, moreover, corroborated the work done by Young.[8c] With 0.03 cc. of staphylococcus toxin, epithelial proliferation reached as high a peak as it did with the stronger dose. On the other hand, in the animals which received only 0.01 cc. each this proliferation was little more in evidence than in the series given iodized oil. Thus the results showed that wherever the stimulus was sufficiently strong and acted for a brief period of time the epithelial response went through a cycle lasting about eight days, waxing to the height of proliferation by the third or fourth day and thereafter regressing.

COMMENT

It should be emphasized again that for practical purposes alveolar epithelium is not visible in ordinary histologic preparations of normal tissue. However, when stimulated with sufficient intensity, it proliferated within three or four days, appearing as a lining of cuboid cells. These were not seen singly or in groups of 3 or 4, but formed a continuous layer completely surrounding the alveoli and bordering both sides of the septums. They were seen at that time to be continuous with the lining of the bronchioles and bronchi. When the alveolar epithelium could be seen as an entity, it was apparent that its constituent cells were not phagocytic (fig. 4, *15*). El-Gazayerli[10] demonstrated this well. His work has been corroborated in the present study.

It has been suggested by some workers that what appears to be a lining of low cuboidal epithelium is merely a flattened layer of mesothelial macrophages lined up on the alveolar wall in a resting state. Others have contended that the cells lining the alveolar wall, although of epithelial nature, are themselves phagocytic. During the course of this study typically stained macrophages, loaded with vacuoles containing neutral red or containing an abundance of carmine or trypan blue, were seen to protrude from the alveolar wall. These occurred singly, in groups of 3 or 4 or in rounded bunches. Where no proliferation of alveolar epithelium was evident, these cells might well have been interpreted as forming the alveolar lining. Cappell,[3] apparently considering them to be alveolar epithelial cells, went further in his observations and concluded that these phagocytes do not move outside of the alveolar

epithelium. He arrived at this conclusion by introducing one vital stain into the trachea and another of a different color into the blood stream, killing his animals at the end of one-half hour. The intratracheal dye was seen in phagocytes within the alveoli. The intravenous dye was seen in phagocytes in the interstitial tissues. He concluded that two phagocytic systems were at work. The present study has shown that dyes introduced intravenously and intratracheally may become well mixed on either side of the epithelial border, given sufficient time and conditions favoring activity of macrophages. It should not be assumed that return of macrophages from alveoli to the interstitial tissues is taken to be the usual course of events. On the contrary, it appears that the phagocytes usually are expectorated along with the foreign substance which they have ingested. This is the course taken by motile entities that find their way into alveoli, as has been so long demonstrated to be true for those parasites that migrate from the blood stream into the lungs and are then expectorated or swallowed. However, when the usual path of least resistance is impeded, as it might be when the alveoli are choked with an exudate, it appears that the macrophages may wander back along the path from which they came.

It is evident from the present study and from the work of others (Gardner and Smith;[7] El-Gazayerli;[10] Wright;[21] Ungar and Wilson[22]) that the macrophages do not arise from the alveolar epithelium. Foot at one time held that they might arise from the capillary endothelium of the lungs. In subsequent work he[2] became convinced that this is not the case and reversed his former stand. Nevertheless, in every section used in the present study a careful examination of the endothelium was made. In no instance was there any indication of transition from endothelial cells to macrophages. Maximow[34] described transformation of lymphocytes and monocytes into macrophages in tissue cultures. Forkner[35] worked with fixed tissues of rabbits vitally stained with neutral red. He described monocytes as being present in all stages of development in all the lymph nodes of the body except the large mesenteric group. With regard for previous work of this nature, in the present investigation a careful study was made of lymphoid accumulations and lymph nodes within the sections. Usually a few mononuclear phagocytic cells were found within each. However, it is to be emphasized that nowhere was there seen anything to suggest transition from lymphocytes to macrophages. As the result of studies of cells supravitally stained with neutral red and janus green, Sabin, Doan and Cunningham[36] concluded that two types of mononuclear phagocytic

34. Maximow, A. A.: Proc. Soc. Exper. Biol. & Med. **24**:570, 1927.

35. Forkner, C. E.: J. Exper. Med. **52**:385, 1930.

36. Sabin, F. R.; Doan, C. A., and Cunningham, R. S.: Contrib. Embryol. (no. 82) **16**:125, 1925.

cells originate in the connective tissues. These they termed monocytes and clasmatocytes. In view of their work, in the present study close attention was paid to the cells in the connective tissues of the lungs. Especially in those sections where the reaction was of considerable intensity, numbers of macrophages were seen in the loose connective tissues about the larger bronchioles, bronchi and blood vessels, particularly the veins. However, so many of the cells were intermediate between the forms containing fine brick red vacuoles of neutral red situated in a rosette at the nuclear hof (Sabin's monocyte) and those larger cells the cytoplasm of which contained an abundance of neutral red vacuoles of various sizes, shades and tints distributed throughout the cytoplasm, with little or no suggestion of rosette arrangement (Sabin's clasmatocyte), that these two were considered morphologic variants of the same cell. This interpretation is consistent with Maximow's opinion concerning monocytes and falls in line with work done by Hetherington and Pierce,[37] who observed transformation of monocytes into macrophages (Sabin's clasmatocytes) and epithelioid cells in tissue cultures of buffy coat. Following Metchnikoff,[38] these cells have been referred to in this paper as macrophages. They are considered to be connective tissue wandering cells, and it should not be assumed that any specific locale is designated as being their site of origin.

The Langhans type of giant cells (fig. 4, *16* and *17*) (Forkner [39]) which were so frequently seen in the lungs after the introduction of staphylococcus toxin apparently did not arise in order that larger quantities of foreign substance might be handled. This opinion was supported by the presence of many such giant cells in the reactions to toxin, whereas they were very little in evidence after the introduction of iodized oil. It seemed obvious that the reaction occasioned by the introduction of iodized oil into the lungs was mainly concerned with removing a comparatively harmless foreign body, whereas in the case of toxin the reaction was a matter of inflammation and repair after damage done to the tissues by a toxic substance.

Finally, with regard to the two types of cell discussed in this paper, a word might be said as to the general function of each. It seems likely that the difference between complete resolution and scarring after inflammatory reactions in the lungs depends on the extent to which pulmonary epithelium is damaged or destroyed. When the strength of staphylococcus toxin is increased over that used here, it is customary to

37. Hetherington, D. C., and Pierce, E. J.: Arch. f. exper. Zellforsch. **12**:1, 1931.

38. Metchnikoff, É.: Lectures on the Comparative Pathology of Inflammation, translated by F. A. Starling and E. H. Starling, London, Kegan Paul, French, Trübner & Co., 1893.

39. Forkner, C. E.: J. Exper. Med. **52**:279, 1930.

see necrosis and subsequent scarring of the tissues. Robertson and Uhley [40] studied the cellular changes which occur in lobar pneumonia. This disease is attended with an infection of the blood stream, as a result of which the capillary walls must sustain severe toxic damage, which becomes manifest during the stage of red hepatization. Consequently the pathologic picture is even more complex than that seen in the material described in this paper. However, they noted that with the appearance of phagocytic macrophages the pneumococci decreased in numbers and that resolution of the pneumonic process followed. Thus the pulmonary epithelium is considered to maintain a surface and the macrophages to act as a defense against toxic and nontoxic foreign substances.

SUMMARY

In these experiments vital and supravital staining technics have been used in the study of cellular reactions consequent to the introduction of both nontoxic and toxic substances into the lungs of rabbits. It has been shown that two distinct types of cells were stimulated. One of them was a nonphagocytic cell lining the alveoli, similar to and continuous with the epithelial cells of the bronchioles. The other type of cell was the macrophage.

The first type arose from the epithelial cells which line the alveoli. It was stimulated slightly by nontoxic substances but was increased to an appreciable extent only when toxic material was injected.

The other type of cell, the macrophage, was stimulated both by the nontoxic and the toxic substances. It has been shown to arise from the connective tissue macrophage and to migrate freely across the alveolar epithelial borders.

40. Robertson, O. H., and Uhley, C. G.: J. Clin. Investigation **15**:115, 1936.

EXPERIMENTAL MUSCULAR DYSTROPHY IN THE GUINEA PIG

A NUTRITIONAL MYODEGENERATION

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AND
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In previous studies we reported on the roles played by activity [1] and by the peripheral nerves [2] in the maintenance of the trophic state of skeletal muscle. It was shown that the mild changes which occur in atrophy of skeletal muscle due to simple disuse are distinctly different from the degenerative ones observed in atrophy following peripheral nerve section. The objective of the present study was to investigate the nutritional factors concerned in maintaining the normal structure and activity of skeletal muscle.

It is well known that inanition or long-continued fasting gives rise to structural changes in skeletal muscles. These changes have been considered "primary" and often occur before any obvious lesions can be observed in other organs. The histologic changes in the hibernating frog have been described by Kremer [3] and those in the eel by d'Ancona.[4] The latter described fading of the cross striations and of the Q disks and disappearance of sarcosomes. He held that these elements are chiefly substances used to yield energy during starvation. The M and Z lines and the sarcolemma were found to be resistant. Myers and Fine [5] found that the pathologic changes occurring in rabbits starved for a long period of time are associated with changes in creatine content, an increased amount of creatine appearing in the urine.

Pathologic changes in skeletal muscle occur with certain vitamin deficiencies. Hyaline necrosis of muscle fibers has been observed in

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This work was carried on with the assistance of a grant from the Council on Physical Therapy of the American Medical Association.

1. Chor, H., and Dolkart, R. E.: Am. J. Physiol. **117**:626, 1936.
2. Chor, H.; Dolkart, R. E., and Davenport, H. A.: Am. J. Physiol. **118**: 580, 1937.
3. Kremer, J.: Virchows Arch. f. path. Anat. **274**:729, 1929-1930.
4. d'Ancona, U.: Am. J. Anat. **39**:168, 1927.
5. Myers, V. C., and Fine, M. S.: J. Biol. Chem. **15**:283, 1913.

experimental scurvy in guinea pigs (Hojer [6]; Meyer and McCormick [7]; Dalldorf [8]). Numerous workers have reported muscle lesions appearing in laboratory animals following administration of vitamin-deficient diets. Goettsch and Pappenheimer [9] reported on muscle lesions in guinea pigs and rabbits on a special prepared diet, complete except for vitamin E, described as progressive dystrophy of the voluntary muscles. Addition of vitamin E to the special diet, however, did not prevent development of the lesions. Hence the lack of this vitamin could not have been the sole factor responsible for the muscle changes. They ruled out such factors as starvation, infection and scurvy. Therefore, some unknown factor in their special diet was considered responsible for the muscular lesions. Morgulis and Spencer [10] repeated this work, using the Goettsch-Pappenheimer diet 13, and obtained similar results. They stated, however, that they were able to prevent as well as cure the "dystrophy" by feeding the following supplements along with the dystrophy-producing diet 13: fresh green alfalfa, lettuce and vitamin E (wheat germ oil), or dry alfalfa and vitamin E (wheat germ oil), or whole wheat germ. They found that the muscles showed progressive regeneration with ultimate restoration of the normal histologic structure.

Madsen, McCay and Maynard [11] reported similar muscular changes in guinea pigs, rabbits, goats and sheep following administration of cod liver oil with normal and synthetic diets. On the basis of their investigations they concluded that the muscle lesions result from some toxic factor in the cod liver oil. Substitution of a vitamin A-D concentrate delayed the onset or lessened the severity of the degenerative changes but did not eliminate them. Reed [12] commented on the histologic changes in skeletal muscle as well as in other tissues of the body in dogs following administration of a high concentration of vitamin D. He concluded that the degenerative changes observed in the tissues were due directly to the toxicity of vitamin D in large doses.

METHODS AND MATERIAL

Fifty guinea pigs weaned at the age of 21 days were used in the present study. After being weaned, they were kept for ten days on a carefully controlled adequate normal diet. In order that the effects of the various dietary factors might be studied, the animals were divided into six groups. The feeding experiments were run simultaneously, and all animals were kept in the same room and exposed to the same changes of temperature.

6. Hojer, A.: Brit. J. Exper. Path. **7**:356, 1926.

7. Meyer, A. W., and McCormick, L. M.: Studies on Scurvy, Stanford University Publications, University Series, Medical Sciences, Stanford University, Calif., Stanford University Press, 1928, vol. 2, no. 2.

8. Dalldorf, G.: J. Exper. Med. **50**:293, 1929.

9. Goettsch, M., and Pappenheimer, A. M.: J. Exper. Med. **54**:145, 1931.

10. Morgulis, S., and Spencer, H. C.: J. Nutrition **11**:573, 1936.

11. Madsen, L. L.; McCay, C. M., and Maynard, L. A.: Memoir 178, Cornell University, Agricultural Experiment Station, 1935.

12. Reed, C. Q.: J. A. M. A. **102**:1745, 1934.

Group 1. Animals 1-5 received 25 Gm. daily of the following normal diet:

Constituent	Parts
Alfalfa meal	15
Casein	5
Calcium carbonate	1
Sodium chloride	1
Butterfat (added daily)	3
Orange juice—3 cc. daily	
Fresh raw carrots daily	

Group 2. Animals 6-14 received 25 Gm. daily of the synthetic diet described by Madsen, McCay and Maynard.[11]

Constituent	Parts
Regenerated cellulose[13]	20
Corn starch	40
Casein	15
Sucrose	10
Yeast	5
Lard	4
Salt mixture[14]	4
Orange juice—3 cc. daily	
Carotene[15]—1 drop daily	
Brewers' yeast—0.2 Gm. added daily for each animal	

Group 3. Animals 15-23 received the normal diet as given group 1 and, in addition, 0.55 cc. of cod liver oil daily.

Group 4. Animals 24-32 received the synthetic diet as given group 2 and, in addition, 0.5 cc. of cod liver oil daily.

Group 5. Animals 33-41 received the synthetic diet as given group 2 and, in addition, were given daily an amount of viosterol[15a] containing 500 U. S. P. units of vitamin D.

Group 6. Animals 42-50 received 25 Gm. daily of the diet described by Goettsch and Pappenheimer:[9]

Constituent	Parts
Rolled oats (Quaker)	355
Wheat bran (Pillsbury)	180
Skim milk powder (Merrell and Soule)	75
Lard	80
Calcium carbonate	15
Sodium chloride	10
Cod liver oil—0.5 cc. daily	
Orange juice—3 cc. daily	

13. This product was purchased from the Sylvania Industrial Corporation, New York. It is a purified form of cellulose, washed free from glycerin. Before being used, the preparation was finely ground in a Wiley mill.

14. The salt mixture—used by Madsen, McCay and Maynard—was composed of:

	Parts		Parts
K_2CO_3	219.0	HCl	28.5
Na_2CO_3	61.0	$CaSO_4$	0.2
$CaCO_3$	192.0	KI	0.03
$MgCO_3$	96.6	$MgSO_4$	9.12
Ferric citrate	20.0	NaF	0.372
$FeSO_4$	30.0	$K_2Al_2(SO_4)_4$	0.037
H_3PO_4	41.8	Citric acid	546.1

15. The carotene used in this investigation was supplied by the S. M. A. Corporation, Cleveland.

15a. The viosterol used in this investigation was supplied by the Abbott Laboratories, North Chicago, Ill.

Explanation of Figure 1

Group 1. All animals in this group showed a consistent regular gain in weight. All survived the experimental period of eighty days. On gross and microscopic examination of the autopsy specimens, no abnormalities were noted.

Group 2. Only 2 of the 9 animals survived the eighty day period. The majority of the animals ultimately succumbed to infections of the respiratory tract. Autopsy revealed marked distention of the gastrointestinal tract, with petechial hemorrhages covering the serosal surface of the large intestines in many instances. The skeletal muscles showed no gross abnormalities.

Group 3. The animals in this group showed a less regular curve of growth than the animals in group 1, which did not receive the cod liver oil supplement. Two animals did not survive the experimental period. The skeletal muscles were extremely pale on gross examination. The original observations of Freeman and Farmer (*Am. J. Physiol.* **113**:1, 1935) concerning the presence of distended gallbladders in animals receiving large doses of cod liver oil were confirmed.

Group 4. The animals in this group did not respond favorably to the synthetic diet plus cod liver oil. None lived more than thirty days, and the majority died between ten and twenty-five days, after the beginning of the experiment. Three of the animals had profuse diarrhea for three to four days preceding death. One animal had bloody feces. Consolidation of the lungs resembling bronchopneumonia in man was observed in practically all of the animals in this group. As in group 3 the gallbladders were distended. Two of the animals showed extreme weakness of the hindlimbs several hours before death.

Group 5. One animal survived the eighty day experimental period. The majority showed a response similar to group 2 with the exception that the average life span was slightly longer. In the animals dying before the expiration of the experimental period pneumonic changes were the predominant pathologic features. As in group 4, several animals showed signs of irritation of the gastrointestinal tract. One showed weakness of the extremities so severe as to render its hind extremities powerless.

Group 6. The animals in this group manifested pathologic changes similar in character to those observed in groups 4 and 5. There was, however, little evidence of gastrointestinal irritation. Three animals showed extreme muscle weakness prior to death. Grossly the muscles appeared somewhat paler than normal. This was especially true in those animals dying of infections of the respiratory tract.

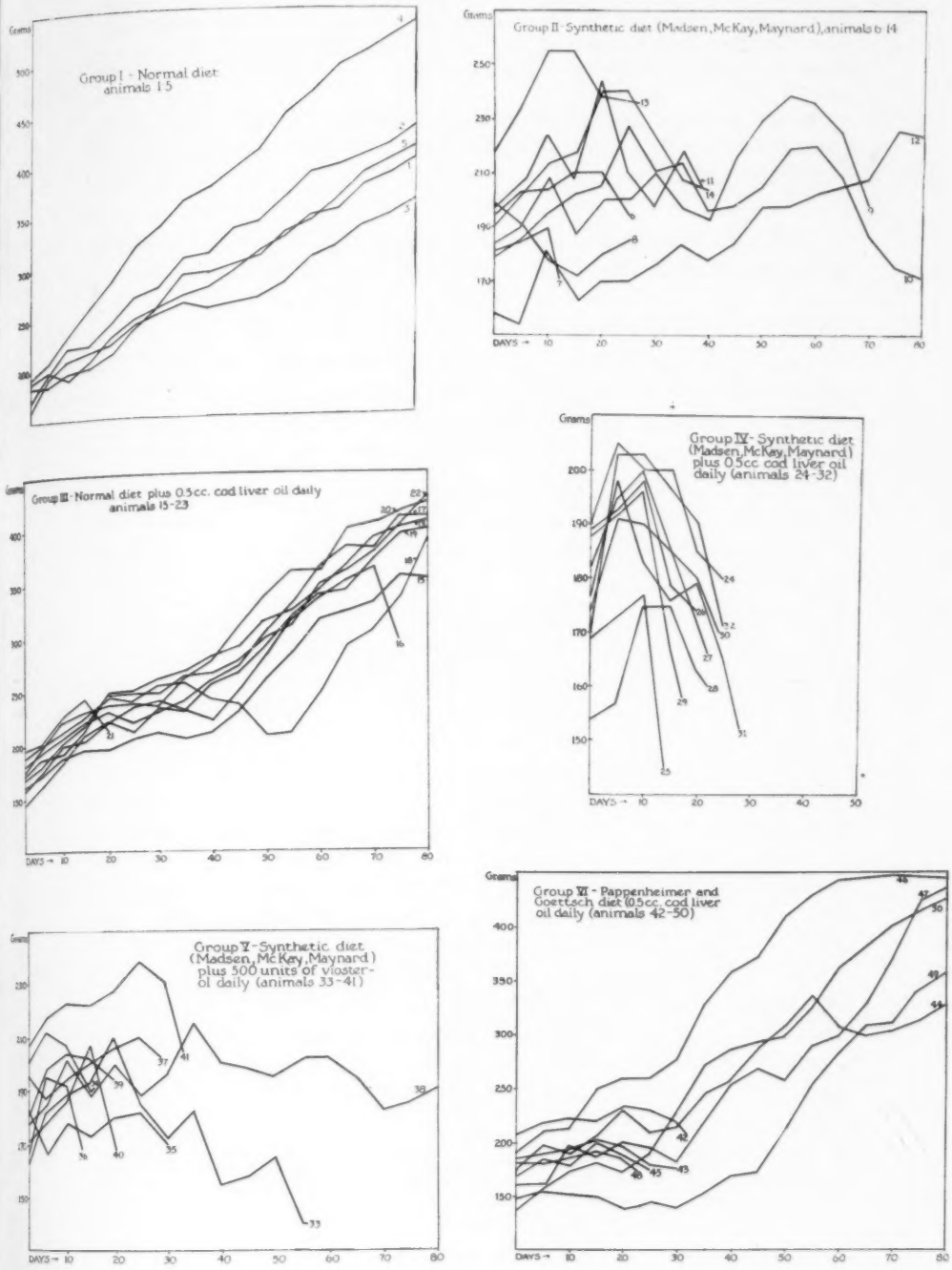
Group I - Normal diet animals 1-5
Group II - Synthetic diet (Madsen, McKay, Maynard), animals 6-14
Group III - Normal diet plus 0.5cc. cod liver oil daily animals 15-23
Group IV - Synthetic diet (Madsen, McKay, Maynard) plus 0.5cc cod liver oil daily (animals 24-32)
Group V - Synthetic diet (Madsen, McKay, Maynard) plus 500 units of viosterol daily (animals 33-41)
Group VI - Pappenheimer and Goettsch diet 0.5cc. cod liver oil daily (animals 42-50)
Grams
DAYS →

Figure 1

In addition to the foregoing animals, a special group of 9 guinea pigs 21 days old was used to ascertain the effect of starvation on muscle. These animals were given submaintenance amounts of an adequate diet. The amounts fed were progressively reduced so that the animals succumbed within fourteen to twenty-one days.

For the histologic studies of the skeletal muscle, the sections were stained by a modified Ranson [15b] pyridine-silver, the hematoxylin-eosin, the Van Gieson and the Bodian [15c] method.

RESULTS

The growth curves for the respective groups of experimental animals clearly demonstrate the response to the feedings of the various diets.

EXAMINATION OF SKELETAL MUSCLES

The gastrocnemius-soleus muscles were removed for examination when the animals were dying or immediately after death. The control animals all survived the experimental period of eighty days and were killed for examination.

Group 1—Normal Diet—Guinea Pigs 1-5.—All specimens were healthy and normal in appearance. Microscopically, there was no trace of any alteration in the muscle fibers, intramuscular nerves or blood vessels.

Group 2—Synthetic Diet (Madsen, McCay and Maynard)—Guinea Pigs 6-14.—Grossly, most of the muscles appeared normal. Microscopically, in some specimens there were irregularly scattered areas of damaged fibers. Many appeared swollen and granular. In these fibers the transverse striations were no longer visible. Instead a homogeneous dark-staining material caused the sarcolemma sheaths to bulge. Only small portions of individual fibers showed this necrosis, the remainder of the fiber being well striated and normal in appearance. In other portions, however, several adjacent fibers were necrotic throughout. A striking feature was the marked nuclear reaction. Many fibers were filled with several types of nuclei. Some were narrow and darkly staining, undoubtedly sarcolemmal; others were vesicular and resembled muscle nuclei; in addition, a great many irregular forms, phagocytic cells, could be distinguished. The process of myophagocytosis was present particularly in the severely necrotic areas, in some instances the degenerated fiber being completely replaced by a mass of these nuclei. There was also a proliferation of connective tissue cells in the areas of degeneration.

In some specimens there was a marked nuclear reaction without degeneration of the fibers. This early nuclear response is characteristic

15b. This stain was described by Chor.[16]

15c. Bodian, D.: Anat. Rec. **65**:89, 1936.

of the reaction of striated muscle under pathologic conditions. These nuclei were largely sarcolemmal and muscle nuclei. The phagocytic reaction occurred later.

Group 3—Normal Diet and 0.5 Cc. Cod Liver Oil Daily—Guinea Pigs 15-23.—The muscles of this group were shrunken and pale with whitish streaks in some of the specimens. They were all extremely flabby. Microscopically, throughout the muscles were large and small areas of severely degenerated fibers in various stages of coagulation necrosis. Usually the damaged fibers were considerably swollen, but in other areas they appeared extremely narrow and atrophic. Increase in nuclei and myophagocytosis were evident.

Group 4—Madsen, McCay and Maynard Synthetic Diet and 0.5 Cc. Cod Liver Oil Daily—Guinea Pigs 24-32.—Many specimens were pale, shrunken and flabby. Microscopically, there were many swollen and degenerated fibers showing vacuoles and myophagocytosis. Multiplication of muscle and sarcolemmal nuclei was present. Little capillary reaction and only moderate infiltration with fibrous tissue were noted.

Group 5—Madsen, McCay and Maynard Synthetic Diet and Viosterol (500 Units)—Guinea Pigs 33-41.—Grossly, the muscles were shrunken, flabby and streaky in appearance. Microscopically, there were many areas of severely damaged fibers. Some were greatly swollen and contained a homogeneous granular substance without trace of cross striations. There was marked nuclear reaction and invasion by phagocytic cells. In other areas only portions of muscle fibers showed these degenerative changes, the remainder being healthy and normal in appearance. Many well preserved fibers were intermingled among the damaged ones. There was no prominent capillary reaction, although a few vessels showed endothelial hypertrophy. An increase in fibrous tissue was evident in some portions of the specimens. There was little reaction of the fat constituents.

Group 6—Goettsch and Pappenheimer Diet.—Grossly, the muscles were pale and flabby, with occasional streaks of white shining through. Microscopically, scattered irregularly throughout the specimen were dark-staining, swollen, degenerated fibers. Striations were no longer present in these fibers. The whole fiber was converted into a homogeneous granular material, resembling hyalin. In some of these areas there was an abundance of nuclei of different types. Some were clear and vesicular with little chromatin content; others resembled plasma cells and leukocytes. Muscle nuclei and sarcolemmal nuclei were definitely increased. This nuclear reaction was also present in fibers which were well preserved. This seems to indicate that this nuclear multiplication is a precursor of degeneration.

Group 7—Starvation Diet.—The muscles grossly were pale and flabby. Microscopically, many fibers showed fading of the cross

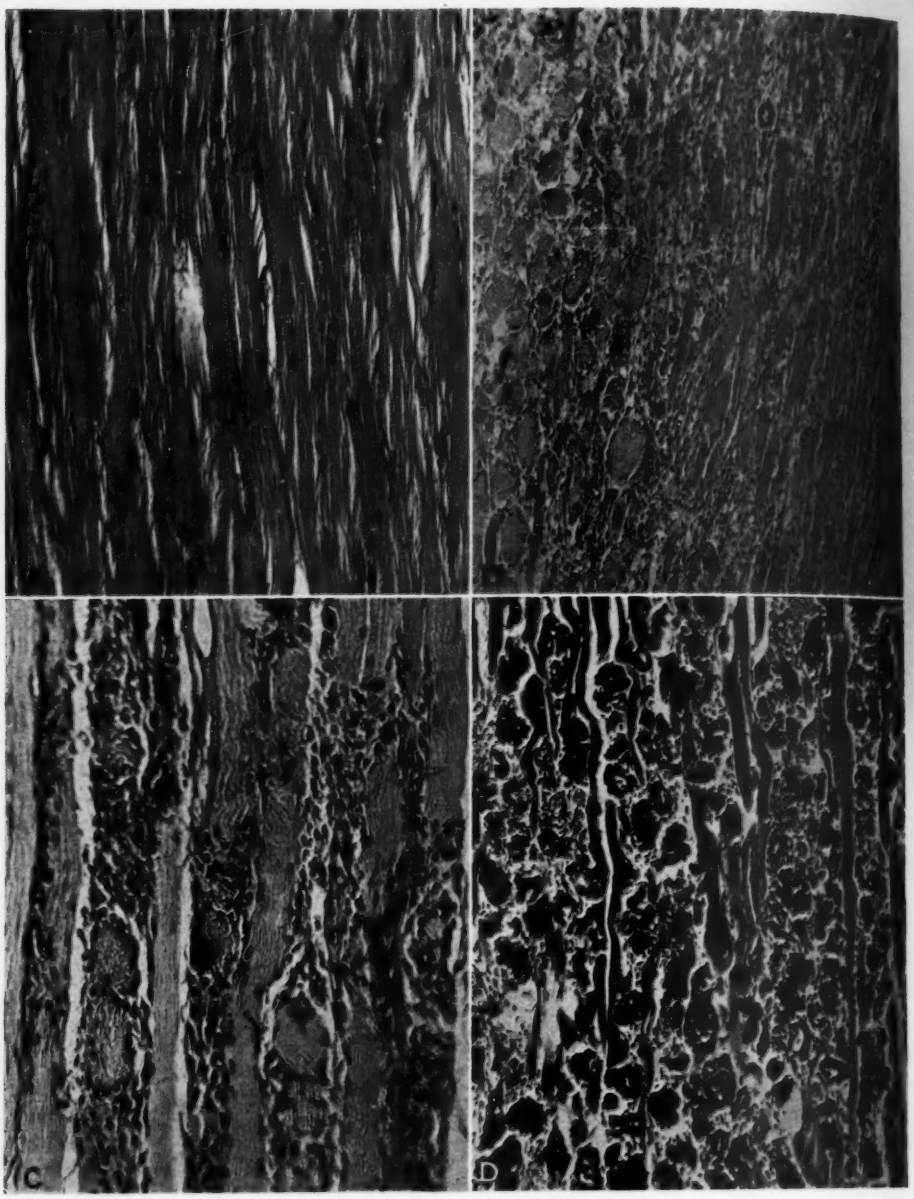

Fig. 2.—*A*, normal skeletal muscle (guinea pig 5); × 295. The fibers are of uniform size, are well striated and show a normal distribution of muscle and sarcolemmal nuclei.

B, "nutritional myodegeneration" (guinea pig 9—Madsen-McCay-Maynard diet); × 135. Many fibers are swollen and show waxy, hyaline degeneration. Some are completely necrotic and covered with nuclei of different types. Other fibers are extremely narrow and atrophic.

C, nutritional myodegeneration (guinea pig 44—Pappenheimer-Goettsch diet); × 250. Many fibers are in a state of coagulation necrosis, with invasion of degenerated fibers by nuclei and phagocytic cells. It may be seen that portions of the fibers appear well striated. This picture resembles closely the degeneration of the rectus abdominis muscle in typhoid fever and pneumonia. (See Forbus,[21] p. 323.)

D, nutritional myodegeneration (guinea pig 16—normal diet and cod liver oil). There are severe coagulation necrosis and degeneration of muscle fibers, in the midst of which may been seen well striated fibers.

Fig. 3.—*E*, nutritional myodegeneration (guinea pig 17—normal diet and cod liver oil). Well preserved intramuscular nerves and terminals in the midst of severely degenerated muscle fibers. This is in marked contrast to "neurogenic" atrophy in which nerve endings and intramuscular nerves show degeneration early before the muscle fibers break down.

F, nutritional myodegeneration (guinea pig 38—Madsen-McCay-Maynard diet and viosterol). Muscle spindle undergoing degenerative changes similar to those taking place in the extrafusal fibers. Note, however, the well preserved intramuscular nerves in the vicinity.

G, normal guinea pig muscle (guinea pig 5), showing normal motor nerve ending on well striated healthy fiber.

H, nutritional myodegeneration (guinea pig 17—normal diet and cod liver oil). Note irregular swellings on the terminal nerve fibers, which are still well preserved, supplying muscle fiber which is severely degenerated.

striations. These fibers were narrow and pale but showed no definite necrosis or degeneration. There was no appreciable increase in nuclei. The blood vessels and the intramuscular nerves and their endings appeared normal. Fat cells were not found in any of the specimens.

THE MOTOR NERVE ENDINGS

The status of the motor nerve endings in various muscle disorders has received but little attention. This no doubt is due to difficulties in staining. In a previous publication [16] a satisfactory method was described, in which a silver-pyridine stain is used. In the present study the Bodian staining method also has given excellent results. Changes in the motor nerve endings have been studied after nerve section and in experimental poliomyelitis. After nerve section degenerative changes are evident within twenty-four hours. These are ushered in by irregular swelling and staining of the fine terminal twigs of the neurofibrillar end brush. By the second day fragmentation of the neurofibrils appears, and the axon and myelin sheath outside the muscle fiber show irregularities in contour and in staining qualities. After the third day the ending is replaced by a granular strand which goes on to complete absorption, and the more proximal portions of the motor nerves show advanced fragmentation of axons and segmentation of myelin. Subsequently only remnants of nerve endings can be made out. These changes characterize "secondary" degeneration.

Kura and Kamesawa [17] studied the motor nerve endings in mice innoculated with tetanus. They found swelling of the axis-cylinders, irregularity of the margins of these and an increase in tortuosity of the end ramifications. They emphasized the fact that the changes in the nerve endings which they described were primary changes and were noticeably different from the changes observed in secondary degeneration. Woolard [18] studied the motor nerve endings in experimental beriberi. He found that the endings were swollen and bulbous and had lost their finer differentiation. He noted that the changes were most marked in the nerve endings and in the myelin and then in the axis-cylinder. These findings suggested that the disease exerts its effect at the peripheral endings.

The finding of well preserved intramuscular nerves and nerve endings in the midst of severely degenerated muscle is good evidence that the muscle changes are not secondary to damage to the lower motor neurons. In atrophy following nerve section and in poliomyelitis when the muscle degeneration is even moderate the nerve endings and intramuscular nerves are no longer to be seen.

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16. Chor, H.: Arch. Neurol. & Psychiat. **29**:344, 1933.
17. Kura, N., and Kamesawa, S.: Tr. Jap. Path. Soc. **18**:330, 1928.
18. Woolard, H. H.: J. Anat. **61**:283, 1927.

Rogers, Pappenheimer and Goettsch [19] studied the motor nerve endings in experimental "muscular dystrophy" in the guinea pig and found no alteration in the peripheral nerves or in the motor terminals. They studied also the larger peripheral nerve trunks and the central nervous system, which were found to be normal. They concluded, therefore, that the muscle changes were primary.

In the present study the intramuscular nerves were found to be well preserved in the midst of severely degenerated muscle. In some specimens, however, the terminal twigs of the telodendrion showed irregular swellings. Fragmentation or disintegration, however, was not found. The slight changes were apparently focal and were either the result of direct action of some toxic agent within the degenerating muscle fibers or a primary effect caused by some factor in the diet. This type of muscle lesion may be considered, therefore, to be extraneural in origin.

COMMENT

The nature of the histologic changes in skeletal muscle which occur in laboratory animals due to dietary factors differs greatly from those occurring as the result of lesions of the motor nervous system. The earliest reaction shows some similarity in that a multiplication of nuclei is the prominent feature in both. Later, however, the changes are quite distinct. The atrophic and degenerative changes in the muscle fibers cut off from their motor nerve consist in shrinkage of the fibers and a granular breakdown of the sarcostylic elements, with replacement by fibrous tissue and fat. The intramuscular blood vessels also react by fibrosis of the vessel walls and endothelial hypertrophy. The intramuscular nerves and their endings completely disappear early in the process. The pathologic changes which result from deficiency in diet and from excess of cod liver oil are those of coagulative necrosis. Many fibers are voluminous and waxy in appearance. Some are narrow and shrunken. Fibrosis is not so prominent, and fat cells are rarely observed. No specific changes in the blood vessels can be detected.

It is interesting to note that in the groups of animals in which muscle lesions were observed the pathologic pictures were similar despite the different nutritional factors which produced them. The lesions obtained with the diet of Pappenheimer and Goettsch and with that of Madsen, McCay and Maynard were duplicated by the administration of cod liver oil to animals receiving a normal diet and by the addition of viosterol or cod liver oil to the synthetic diet of Madsen, McCay and Maynard. Rogers, Pappenheimer and Goettsch emphasized the fact that despite extensive degeneration of the muscle tissue the intramuscular nerves and their endings persist. Our observations are in agreement, although

19. Rogers, W. M.; Pappenheimer, A. M., and Goettsch, M.: J. Exper. Med. **54**:167, 1931.

we did find slight changes at the very termination of the motor endings, which we think were due to a focal reaction.

The similarity between the pathologic changes observed in the present study and those described by Zenker [20] in typhoid fever and by Forbus [21] in pneumonia is quite striking. The degenerative changes in pneumonia have been attributed to a toxic factor elaborated by the specific organism.

In our opinion the use of the term "dystrophy" to designate the muscle lesion resulting from nutritional deficiency may be confusing inasmuch as muscular dystrophy in man is a specific syndrome with characteristic clinical features. The word "dystrophy" is derived from the Greek δυσ—"badly" and γρέφω—"nourish." Etymologically, therefore, its usage in reference to conditions of malnutrition is correct. In human muscular dystrophy the muscle fibers show chronic degeneration with considerable replacement by fibrous tissue and fat. The status of the intramuscular nerves and their endings is still poorly understood. The disease occurs usually in childhood and is slowly progressive. There is a selective involvement of definite muscle groups rather than a generalized involvement of the musculature. The familial incidence and transmission through several generations suggest an underlying disturbance of the germ plasm. This is supported by the frequent association with other evidence of constitutional defect and inferiority, such as mental deficiency, epilepsy, optic atrophy, scleroderma and Friedreich's ataxia.

Because of certain similarities in the histologic and chemical aspects of these muscle lesions, there has been a tendency to apply the information obtained from experimental studies to the interpretation and treatment of human muscular dystrophies. We therefore suggest the use of the term "nutritional myodegeneration" rather than "dystrophy" for the muscle lesions produced by dietary alterations.

SUMMARY

Degenerative changes in skeletal muscle of guinea pigs have been produced by synthetic diets previously described by Pappenheimer and Goettsch and by Madsen, McCay and Maynard. These lesions were duplicated by the administration of cod liver oil to animals receiving a normal diet and by feeding animals the synthetic diet of Madsen, McCay and Maynard plus viosterol or cod liver oil.

The pathologic changes are those of coagulative necrosis. They closely resemble those described by Zenker in typhoid fever and by Forbus in pneumonia.

20. Zenker, F. A.: Ueber die Veränderungen der willkührlichen Muskeln im Typhus abdominalis, Erlangen, A. E. Junge, 1863.

21. Forbus, W. D.: Arch. Path. **2**:318, 1926.

Despite marked degeneration of the muscle fibers, the intramuscular nerves and their terminals are well preserved. These observations are in agreement with those reported by Rogers, Pappenheimer and Goettsch. This type of muscle lesion may be considered, therefore, to be extraneural in origin.

The histologic alterations characteristic of this experimental myopathy are different from those observed in muscular dystrophy in man. We suggest the use of the term "nutritional myodegeneration" rather than "dystrophy" for the muscle lesions produced by dietary alterations.

EFFECTS OF ORGANISMAL DIFFERENTIALS ON THE DISTRIBUTION OF LEUKOCYTES IN THE CIRCULATING BLOOD

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NATURE OF THE PROBLEM

Two types of individuality may be distinguished according to Loeb:[1] (1) the mosaic type and (2) the type based on the presence of organismal differentials.

Each individual, with the possible exception of a unioval twin, differs from all others in a great variety of characteristics localized in the various tissues and organs of the body. These differences are structural as well as chemical and functional. Each individual is a mosaic of tissues and organs characterized by individual differences.

In addition, each individual organism, particularly if it belongs to the higher classes of animals, possesses a chemical characteristic which is common to all that individual's tissues and organs and differentiates it from all other individuals of the same species, each one of which also is distinguished by such a chemical denominator of the various tissue and organ constituents. These chemical characteristics distinguishing each individual of the same species have been designated by Loeb as individuality differentials. Correspondingly, species, orders and classes may be distinguished by their species, order and class differentials. All these various kinds of differentials, including the individuality differentials, comprise the organismal differentials. In an extensive series of transplantations carried out by Loeb and his associates[2] it could be

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These experiments formed the basis of a dissertation presented to the Board of Graduate Studies of Washington University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

1. Loeb, L.: Science **86**:1, 1937.

2. (*a*) Hesselberg, C., and Loeb, L.: J. M. Research **38**:33, 1918. (*b*) Loeb, L.: ibid. **6**:28, 1902; (*c*) **8**:44, 1902; (*d*) **39**:189, 1918; (*e*) **32**:353, 1918; (*f*) **41**:305, 1920; (*g*) Biol. Bull. **40**:143, 1921; (*h*) Am. J. Path. **2**:99, (*i*) 111, (*m*) 301 and (*n*) 315, 1926; (*o*) **3**:45, 1927; (*p*) Arch. Path. **10**:224, 1930; (*q*) Physiol. Rev. **10**:4, 1930; (*r*) Biol. Bull. **68**:440, 1935. (*s*) Loeb, L., and Addison, W. H. F.: Arch. f. Entwcklngsmechn. d. Organ. **27**:73, 1909; (*t*) **32**:44, 1911. (*u*) Loeb, L., and Harter, J. S.: Am. J. Path. **2**:521, 1926. Loeb.[1]

shown that each one of the various types of transplantations—autogenous (transplantation of a piece of tissue into the same organism), syngenesious (transplantation into a nearly related animal), homogenous (transplantation into a nonrelated or distantly related animal of the same species) and heterogenous (transplantation into a different species)—is characterized by modes of reaction against the transplant on the part of the white blood cells, in particular the lymphocytes and polymorphonuclear leukocytes, and the connective tissue cells and blood vessels in accordance with the relationship of host and transplant. It is as if these various tissue elements belonging to the host recognized the genetic relationship between host and transplant and acted accordingly. It is especially the lymphocytes whose mode of reaction serves as the finest indication of individual differences as represented by the individuality differentials of host and transplant.

I shall now discuss briefly the nature of the interactions between host and transplant in accordance with their relationship, because a knowledge of these data is necessary for the understanding of the investigations on which I shall report subsequently. It may be assumed that a diffusion of certain substances from the graft into the host takes place, as a result of which certain kinds of cells of the host display a specific reaction against the transplant.

After autotransplantation lymphocytes are practically lacking around the graft, and connective tissue cells are attracted only to a moderate degree. The blood supply, on the other hand, is good because of the active ingrowth of capillaries into the transplant.

After homotransplantation there occurs around the transplant a reaction in which lymphocytes, connective tissue cells and blood vessels of the host are concerned. Lymphocytes are attracted by the tissue possessing a homodifferential; they collect around it, infiltrate it and injure it. The injury under certain conditions may progress to total destruction. Connective tissue cells also collect in increased numbers around the graft and invade it. Moreover, they tend to form dense hyaline tissue in contrast to their behavior in the autotransplant, where in contact with the transplanted cells they give rise to a cellular-fibrillar structure. The growth of capillaries into the homogenous transplant, and hence the blood supply of the latter, is much diminished in comparison with that which is characteristic of the autotransplant.

The reaction against the syngenesiotransplant stands midway between those observed after autotransplantation and homotransplantation. In comparison with the changes observed after homotransplantation the connective tissue reaction is, on the average, less marked; the lymphocytic reaction likewise is, on the average, diminished, but it varies in different cases in accordance with the genetic relationship between host and transplant. It may approach the autoreaction on the one hand, if the

genetic constitutions are very similar, and the homoreaction at the other extreme. All kinds of intermediate reactions have been observed in the experiments of Loeb.[20] In some cases, however, the diminution in the injurious action of fibroblasts, which is characteristic of syngenesious transplantation, may give the lymphocytes a chance to collect ultimately in larger numbers around the graft than in cases of homotransplantation, in which the marked new formation of fibrous tissue may injure the transplant at so early a stage that the chance of the lymphocytes to accumulate around it is diminished pari passu with the increase in the amount or in the toxicity of the homogenous substance which is able to diffuse into the host.

If the degree of relationship between host and donor is very far removed, as in the case of heterotransplantation, the reactions are more severe. Here the body fluids of the host are so different from those to which the transplanted tissue is adapted that they become markedly toxic for the latter; they become converted into heterotoxins and if the transplant is not very resistant to injuries, cause its death in a relatively short time. The connective tissue reaction of the host is very strong; besides, in an early period after transplantation polymorphonuclear leukocytes are attracted rather than lymphocytes, the latter arriving at a later period when the acutely acting heterotoxins have been largely absorbed.

The lymphocytes are the host cells which are most sensitive to differences between the individuality differentials of living tissues within the same organism. They are therefore very active in cases of syngenesiotransplantation and in cases in which the homodifferentials do not exceed a certain degree of difference. If the differences between the genetic constitutions of homogenous graft and host are very great, the connective tissue reaction tends to preponderate, and it may cause marked injury of the transplanted tissue at an early period. As stated, as a result of this injurious reaction, the lymphocytes do not find a chance to accumulate to the same extent as in the case of syngenesiotransplantation or of transplantation in which the differences between the homodifferentials of host and graft are less pronounced.

The body fluids of the host may also take part in reactions against homogenous tissues; but they are much less prominent in these reactions than in the reactions to heterotransplantation. It is especially the more sensitive tissues which are directly injured by the constituents of the strange homogenous body fluids.

It is clear, then, that the host cells can recognize a strange organismal differential and that, further, they can distinguish between different degrees of relationship between host and transplant. There is, however, a limit to this power of discrimination in that after a certain threshold of strangeness has been reached the reaction is maximal and

cannot be much increased by grafts of tissue from more distant donors This applies to heterotransplantation.

At about the same time that experiments concerning the local reaction of the host against normal transplanted tissues were carried out by Loeb and his collaborators, somewhat comparable observations were made independently with transplanted tumors in rats and mice. Da Fano [3] noticed in mice that after transplantation of a piece of carcinoma of the mammary gland lymphocytes and monocytes accumulated around the graft and at places distant from the tumor. He attributed to these cells the function of initiating immunity against the carcinomatous tissue. Furthermore, Tyzzer [4] and Burgess [5] observed around grafts of strange tumor tissue a collection of various leukocytes and increased activity of fibroblasts. They attributed these reactions to an inflammatory injury of the host tissue surrounding the tumor by anaphylotoxin-like substances which developed in the host as the result of the tumor transplantation. Still another investigator, Baeslack,[6] noticed that if a transplanted tumor grows continuously the polymorphonuclear leukocytes in the circulation of the host increase in number, whereas if the tumor retrogresses the lymphocytes increase in number. It was especially Murphy [7] who, several years later, likewise stressed the behavior of the lymphocytes in the reactions against transplanted tumors. Also, according to this investigator, a general increase in the number of these cells is due to the development of immunity against the tumor graft. If the host resists the growth of the tumor and becomes immune, the lymphocytes in the lymph glands multiply more actively, and the lymphocytes in the general circulation increase in number; this does not occur if the host does not become resistant against the tumor and the latter grows successfully. He believed that if by treatment, such as the application of small doses of roentgen rays to the mouse, exposure of the animal to certain degrees of dry heat or the injection of certain oils into the mouse, an increase in the number of lymphocytes circulating in the vascular system is produced prior to transplantation of the tumor, immunity against the implanted tumor is increased. Under these conditions the animal is able to react even against autotransplanted pieces of tumor, i. e., against tissues which were derived from the host. By such means it is also possible to enhance resistance

3. Da Fano, C.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **5**:1, 1910.

4. Tyzzer, E. E.: J. Cancer Research **8**:109, 1916.

5. Burgess, A. W.: J. M. Research **16**:575, 1909.

6. Baeslack, F. W.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **20**:421, 1914.

7. Murphy, J. B.: The Lymphocyte in Resistance to Tissue Grafting, Malignant Disease, and Tuberculous Infection: An Experimental Study, Monograph 21, Rockefeller Institute for Medical Research, 1926.

against infection with tubercle bacilli in animals otherwise susceptible to infection with these organisms. The lymphocyte is then, according to Murphy, the main agent in the establishment of immunity against strange tumors, as well as against tumors derived from the animal's own cells, and also against certain pathogenic micro-organisms.

The majority of investigators assumed that the reactions against tumor grafts were due to immunity developing against the transplanted tissues and furthermore considered this immunity, or resistance, as specific for tumor. Yet the fact that a typical difference in the reactions of the host against the autotransplant and the homotransplant could be shown in the case of transplantation of tumor as well as in that of transplantation of normal tissues (Loeb [2q]) and the fact that normal homogenous tissues produce immunity against homogenous tumors, while immunity against heterogenous tissues can be produced only through previous implantation of normal tissues derived from the same species (Ehrlich;[8] Schoene[9]) suggested that the organismal differentials of normal tissues are also present in tumor tissues (Loeb [2q]). It was therefore conceivable that also after transplantation of normal tissues changes in the constitution of the white blood cells might take place in the circulating blood and that these organismal differentials might diffuse not only into the tissues surrounding the graft but also into the general circulation and from there be carried to the blood-forming organs, where they would cause a stimulation of the lymphocytes or polymorphonuclear leukocytes in accordance with the kind of organismal differential active. I decided to determine, therefore, whether grafting of normal tissues was followed by characteristic changes in the relative and absolute number of white blood cells in the circulating blood corresponding to the local changes which were produced by the transplanted tissue. That diffusion of organismal differentials into the area of the host surrounding the graft takes place was indicated by an experiment of Crossen[10] in which when lymph gland was autotransplanted in the vicinity of homotransplanted cartilage a movement of lymphocytes from the lymph gland to the cartilage could be seen to occur.

MATERIALS AND METHODS

The various tissues used in the experiments were grafted into subcutaneous pockets in guinea pigs, rats, pigeons or chickens as host animals. As a rule, the transplant was small, similar in size to a single lobe of the thyroid of a

8. Ehrlich, P.: Ztschr. f. ärztl. Fortbild. **3**:211, 1906; Arb. a. d. k. Inst. f. exper. Therap. zu Frankfurt a. M. **1**:77, 1906.

9. Schoene, G.: Verhandl. d. deutsch. Gesellsch. f. Chir. **36**:213, 1907.

10. Crossen, R. J.: Arch. Path. **6**:396, 1928.

guinea pig or rat. However, in some cases which I shall mention, multiple pieces were grafted simultaneously or in succession. Besides tissues, various nonliving substances were either implanted into subcutaneous pockets or injected in the form of solutions into guinea pigs and rats. Before and at various intervals after the transplantation, in the majority of cases every second day, differential counts of the white blood cells were made. In addition to the differential counts, total counts were made in a certain number of animals bearing autogenous, homogenous or heterogenous grafts.

In still other experiments excised organs were exposed to variations in temperature and to certain chemical substances for stated periods of time previous to transplantation in order that the effects of these factors on the organismal differentials of the transplanted tissues might be observed.

The differential counts were made by the dry smear method. A drop of blood from either the ear vein (guinea pig), the tail vein (rat) or a small superficial mammary vein (pigeon and chicken) was drawn out on a clean slide. This preparation was stained by the Wright blood stain method. Invariably 300 or more white cells were counted in each case.

Total counts were made with the Spencer Brite-Line hemocytometer according to the method described by Todd and Sanford.[11] Since eosinophils and basophils were present in very small numbers and were apparently unaffected by the experimental procedures, they were grouped with the neutrophils as polymorphonuclear leukocytes. It should be noted here that when the term "leukocytes" is used it is as a synonym for white blood cells and comprises lymphocytes as well as polymorphonuclear leukocytes.

CONTROLS

Differential counts made daily for from three to five days preceding transplantation served as control values. If the lymphocytes were the cells which showed an increase after grafting of a tissue, the highest percentage of lymphocytes found during the control period was taken as the base line for determining the effects exerted by the experimental procedures.

Certain extrinsic factors which are known to exert a considerable influence on the blood picture in both man and higher animals were taken into account both in arriving at control values and in interpreting the changes to be described.

In a series of experiments embracing 33 guinea pigs, 42 rats and 33 pigeons, differential counts were made on animals of various ages. The results are shown in table 1. It should be noted that as far as could be determined by gross examination these animals were free from infection.

From these results it appears that very young animals have greater lymphocyte counts than middle-aged ones. This comes out clearly in the case of guinea pigs in which it is seen that recently born animals have from 11 to 16 per cent more lymphocytes than young adults. In still older animals there is an increase. There seems to be a tendency toward increase of lymphocytes again in the peripheral circulation in pigeons and guinea pigs as these animals grow old. However, since only a relatively small number of old individuals of these three species could be obtained and since a corresponding curve was not observed in the case of rats, it may be that the variations in some of these cases were accidental.

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11. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis by Laboratory Methods: A Working Manual of Clinical Pathology, ed. 8, Philadelphia, W. B. Saunders Company, 1935.

It seemed that for the purpose of the experiments in view the most favorable age of the host animals in each of the three species was that of young adults. Rats weighing from 80 to 120 Gm. and guinea pigs weighing from 300 to 500 Gm. belong approximately to this class. The weight of the pigeons varied between 350 and 500 Gm. By carefully checking the weights of the animals which were used, it was possible to keep the age factor fairly constant as far as the host animals were concerned.

It is well known that leukocytosis follows ingestion of food, and there is, furthermore, general agreement that the maximum rise in the total leukocyte count occurs within two to three hours after a meal and may amount to as much as 100 per cent.

In order to avoid variations in counts due to food intake, specimens were invariably taken in the morning, when a period of approximately eighteen to twenty hours had elapsed since the last feeding.

TABLE 1.—*Influence of Age on Lymphocyte Count*

Animal	Number on Which Counts Were Made	Age	Average Percentage of Lymphocytes
Pigeon	10	2 days-4 weeks	69.5
	12	2 mo.-1 yr.	60.2
	10	1½-4 yr.	62.5
	1	5 yr.	69.0
Guinea pig	10	1 day-4 weeks	71.5
	10	6 weeks-6 mo.	55.8
	10	7 mo.-2 yr.	60.3
	3	3-5 yr.	72.0
Rat	12	1-4 weeks	68.3
	12	5 weeks-6 mo.	62.8
	15	7 mo.-2 yr.	58.3
	3	2½-3 yr.	57.0

Temperature effects must also be considered since Murphy and Sturm,[12] subjecting mice to dry heat at 55 to 65 C. for five minutes, found that both neutrophils and lymphocytes fell immediately after the exposure. Subsequently, however, the neutrophils recovered at a slow rate while the lymphocytes increased so rapidly that by the second week after heating the count often reached a point as high as 200 to 300 per cent above normal. I found an increase in lymphocytes in one set of control experiments carried out in the hot weather of early June 1936. These experiments were therefore discarded. All the other experiments were done at temperatures which were within the normal range; in these an increase in the lymphocytes due to a rise in temperature could be excluded.

Recently von Euler and Malmberg[13] made comparative white blood cell counts of blood taken from the heart, the marginal ear vein and "pooled blood" of the guinea pig. They found the count of heart blood to be approximately one-third that of blood from the ear vein and the count of "pooled blood" two-thirds as high as that of blood from the ear vein. I attempted to repeat their experiments by making similar comparative counts of blood from the heart and from the ear vein every other day for a period of eight days. Thus each figure in table 2, which gives a summary of the results obtained, is an average of 4 counts.

12. Murphy, J. B., and Sturm, E.: J. Exper. Med. **29**:1, 1919.

13. von Euler, H., and Malmberg, M.: Naturwissenschaften **24**:713, 1936.

Here, too, there are consistent differences between the values for heart and ear vein blood, but these differences are not nearly as great as those found by von Euler and Malmberg, since the average count of blood from the heart is 90 per cent of the count of blood from the ear vein, as compared with approximately 33.33 per cent found by the latter investigators.

However, it was possible by modifying the mode of procedure to obtain differences between counts comparable to those obtained by von Euler and Malmberg. This modification consisted in allowing the heart blood to stagnate for a short time after its removal from the guinea pig.

Differential counts made simultaneously with the total counts in the same guinea pigs showed that the proportion of lymphocytes in blood taken from the ear vein was higher than that in blood taken from the heart. There were on the average 55 per cent lymphocytes in the heart blood as compared with 62.8 per cent in the blood taken from the ear vein.

TABLE 2.—*Comparative Counts of White Blood Cells in Blood from the Heart and from the Ear Vein*

Guinea Pig	Average Count of White Blood Cells per Cu. Mm. of Blood From Ear Vein	Average Count of White Blood Cells per Cu. Mm. of Blood From Heart
1	8,150	7,250
2	7,900	7,400
3	7,100	6,500
4	7,100	7,000
5	8,600	8,450
6	8,800	6,500
7	6,500	6,150
8	8,100	7,700
9	6,850	6,400
10	7,500	6,550
11	7,700	6,850
12	6,800	5,000
Average	7,591	6,813
	6,813 / 7,591 = 0.90 (90%)	

In order to avoid the difficulties shown by these experiments peripheral blood was used in all counts. As stated, in the case of guinea pigs blood from the marginal ear vein was used; in that of rats blood from the tail vein, and in that of pigeons and chickens blood from a superficial vein of the breast.

Further care was taken to eliminate all animals that were diseased and to discard all experiments in which the transplanted tissue showed any sign of infection.

NUMBER OF WHITE BLOOD CORPUSCLES IN THE CIRCULATION OF NORMAL ANIMALS

With the factors of nutrition, temperature, age and source of blood thus adequately controlled, both total and relative counts were made on a series of normal guinea pigs, while differential counts alone were made on a similar group of rats and pigeons, in order to determine the range of variation in the numbers of leukocytes circulating in the peripheral blood in normal animals that had not been operated on. The differential counts of the pigeon and rat were comparable. Since there is an inverse

ratio between changes in relative couts of lymphocytes and polymorphonuclear leukocytes (the proportion of lymphocytes rises while that of polymorphonuclear leukocytes decreases correspondingly) it is necessary to show only the variations in the number of lymphocytes. The average of the counts made on the 12 guinea pigs the first day represents the base line (O), and increases or decreases found in subsequent counts are measured by the height and position of the ordinates. The average increases and decreases found over a twenty day period were relatively slight. In the case of the lymphocytes the average deviation never exceeded 5 per cent in either direction; the average total counts, similarly, never showed a deviation of more than 500 in the positive and 600 in the negative direction. In only 2 guinea pigs did the increase in lymphocytes exceed 5 per cent; it rose to 8 per cent on the thirteenth day in one and to 10 per cent on the fifteenth day in the other. Similarly, deviations in the total counts greater than 500 in either direction were observed in only 3 animals. One showed an increase of 750 on the third day and the other 2 decreases of 800 and 750 white blood cells on the ninth day.

As far as the range of variations in the differential counts of pigeons and rats is concerned, it was very similar to that noted in guinea pigs.

EFFECTS OF TISSUE TRANSPLANTS ON THE ABSOLUTE AND RELATIVE NUMBERS OF VARIOUS TYPES OF LEUKOCYTES IN THE PERIPHERAL CIRCULATION OF THE HOST

Autotransplants.—In all, 42 experiments were made in which the effect of autotransplantation on the numbers of leukocytes in the peripheral blood was observed—24 with guinea pigs, 12 with rats and 6 with pigeons. Thyroid, testis, ocular lens and blood clot were used. As can be seen in table 3, in no case did the average maximum increase in percentage of lymphocytes exceed 6.5 per cent; if one excepts autotransplantation of blood clots, the average relative increase in lymphocytes was never greater than 4.8 per cent. Similarly, in the case of total counts as determined in 4 guinea pigs following autotransplantation of thyroid, the increase in no instance exceeded 500 white blood cells per cubic millimeter (table 4). However, in 3 single counts increases in percentage of lymphocytes did exceed 10 per cent. Two guinea pigs showed a 12 per cent and a rat a 10 per cent increase. Two of these exceptions occurred on the tenth day, the third on the sixteenth day, and they were probably due to some environmental factor. These high counts were, however, transient, since on the next occasion on which counts were made on these animals they showed an increase of less than 5 per cent.

It might be concluded that the variations found after autotransplantation were similar to those observed in normal animals. This is, however,

not the case, since in these experiments, with few exceptions, negative deviations were not observed. After autotransplantation there is generally an average maximum increase in percentage of lymphocytes of 5 per cent, and the average maximum increase in the total number of white blood cells is 500 leukocytes. In general there is a tendency for the counts to remain at the base line and occasionally to rise slightly above it during the course of a twenty day experiment. If the experiments with pigeons are omitted, only 12 of 378 counts made in the 36 cases of autotransplantation showed negative deviations, and these were

TABLE 3.—*Effect of Autotransplantation on Differential Counts of White Blood Cells in Peripheral Blood*

Animal Species	Experiments*	Tissue Transplanted	Average Maximum Percental Increase of Lymphocytes	Average Day of Maximum Increase	Range of Variation in Percental Increase of Lymphocytes	Range of Variation in Day of Maximum Increase
Guinea pig	12	Thyroid	4.1	6.1	1-12	1-11
	6	Blood clot	6.5	4.3	0-7	2-18
	6	Testis	4.8	5.5	4-6	2-6
Rat	3	Thyroid	3.3	11.0	2-10	5-16
	3	Blood clot	4.7	6.0	4-5	2-9
	6	Eye lens	4.3	5.7	2-7	3-9
Pigeon	6	Thyroid	0	...	...	...

* The total number of experiments was 42. In all the tables cases in which the transplants became infected were excluded.

TABLE 4.—*Effect of Autotransplantation on Total Counts of White Blood Cells in Peripheral Blood*

Animal Species	Experiments	Tissue Transplanted	Average Maximum Increase of White Blood Cells per Cu. Mm.	Average Day of Maximum Increase	Range of Variation in Increase of White Blood Cells per Cu. Mm.	Range of Variation in Day of Increase
Guinea pig	4	Thyroid	275	4.5	50-500	3-9

transient, the counts returning either to normal or to slightly above normal usually at the first, or at the latest at the second, subsequent count. The experiments with pigeons in which thyroid was autotransplanted differed from those with the other species inasmuch as the counts were very similar to those observed in controls, showing both slight positive and slight negative deviations.

Wounds; Inert Foreign Bodies: It was now necessary to determine whether this difference between the normal variations and the variations noted in bearers of autotransplants was due to the presence of the implanted tissue as such or whether it was due to nonspecific factors. Various inert substances, such as pieces of paraffin, silk thread

or agar, were therefore implanted into animals and differential counts made in the same way as in the experiments with autotransplantation. Table 4 shows the results. The positive deviations in animals with implanted inert substances were very similar in range to those in animals with autotransplants. There is, however, one minor exception to this conclusion. In 3 experiments in which paraffin was implanted changes similar to those observed in controls occurred, i. e., both small positive and small negative deviations. In additional experiments cuts were made in the skin of 4 guinea pigs, and these incisions were immediately closed by suture. Differential counts made on these animals were comparable in every way to those made after autotransplantation as well as after implantation of inert substances.

TABLE 5.—*Effect of Implantation of Inert Materials and of Operative Intervention on Blood Counts (Differential Counts)*

Animal Species	Experiments*	Substance Implanted	Average Maximum Percental Increase of Lymphocytes	Average Day of Maximum Increase	Range of Variation in Percental Increase of Lymphocytes	Range of Variation in Day of Maximum Increase
Guinea pig	4	Agar	6.6	10	5-7	8-12
	4	Thread	4.5	6.5	3-6	3-13
	16	Paraffin	3.4	6.8	0-6	1-18
	4	Cut and suture	4.5	5.5	2-8	1-19
Rat	4	Paraffin	2.0	4.5	3.5	1-4

* The total number of experiments was 32.

It is evident, then that simple operative procedures may act as a mild stimulant to the hemopoietic tissues, with the result that instead of observing a curve in the percentage of lymphocytes similar to that seen in controls, which is characterized by small positive and small negative deviations, one sees a number of small increases and almost no decreases. The time when the maximum of lymphocytic increase is observed is variable, ranging in both the case of autotransplantation and that of implantation of inert substances between one and eighteen days. The pigeon, however, appears to be refractory to mild stimulations such as those produced by autotransplantation and by the insertion of inert foreign bodies, since in all experiments of this kind the variations were the same as those previously observed in normal animals; also in 3 guinea pigs implantation of paraffin did not cause an increase in numbers of white blood cells.

One may therefore conclude that no changes in the white cell content of the peripheral blood could be attributed specifically to the presence of the autotransplanted tissue. The reactions observed were the result of nonspecific operative disturbances during the process of transplantation.

Homogenous Transplants.—In 253 experiments the effect of homotransplantation on the numbers of leukocytes was studied. As is shown in table 6, these transplantations included a wide variety of tissues. As usual, grafts were made into subcutaneous pockets with exception of 6 instances in which thyroids were transplanted to muscles in pigeons. Since the changes observed in these 6 cases did not differ from those seen after subcutaneous transplantation, they are included with the latter in table 6.

As can be seen from table 6, with few exceptions, to be discussed later, there is a marked increase in the proportion of lymphocytes in the differential count following homotransplantation. The increase is well above 10 per cent, and the day of maximum change shows much less variation than was found after autotransplantation or after implantation of inert substances.

After homotransplantation of thyroid (108 experiments), it was observed that by the fourth or fifth day there was a clearcut increase in lymphocytes. The maximum increase was usually reached on the sixth to seventh day; this was followed by regression to approximately the normal count. After homotransplantation of blood clot the reaction was more rapid. Definite increases in lymphocytes could be noted as early as the first or second day, while the maximum increase was generally observed on about the fourth day. In the case of the chicken the relative increase in lymphocytes was somewhat less marked and the time of the maximum increase somewhat later than in the other species, owing probably to the fact that the normal percentage of lymphocytes in the chicken is already very high (75 to 85 per cent). Under this condition a stimulus might be expected to be less effective than in the other three species.

Table 6 shows further that the increase in the average percentage of lymphocytes varies from 13.5 per cent in the pigeon to 16.9 per cent and 16.6 per cent in the rat and guinea pig, respectively, following homotransplantation of thyroid. Again, the pigeon generally has a higher normal proportion of lymphocytes than either of the other two species and the relative increase in lymphocytes under this experimental condition is therefore less great in the former.

It is interesting to note that 29 of the 35 experiments in which increases over 25 per cent were exhibited were carried out during the last two years of the investigation, while only 4 of the 35 experiments in which increases less than 25 per cent were shown were done during the same period. This makes it probable that with gradual improvement in technic the counts became slightly higher and that the average increase in lymphocytes in the homotransplantation experiments carried out in the course of three years was actually somewhat greater than the

figures given here indicate; however, these variations are not of a sufficient magnitude to alter the conclusions.

The total counts confirm essentially the results obtained with differential counts. After homotransplantation there is an increase in the total number of white blood cells per cubic millimeter of the peripheral blood, which reaches a maximum on the sixth to eighth day. This coincides approximately with the time at which the maximum increase

TABLE 6.—*Effect of Homotransplantation on the Distribution of Leukocytes in the Circulating Blood (Differential Counts)*

Animal Species	Tissue Transplanted	Experiments*	Average Maximum Percental Increase of Lymphocytes	Average Day of Maximum Increase	Range of Variation in Maximum Percental Increase	Range of Variation in Day of Maximum Increase
Guinea pig	Thyroid	68	16.6	7.1	11-31	4-12
	Liver	16	14.3	7.1	11-20	3-10
	Kidney	6	15.5	7.2	10-27	5-10
	Skeletal muscle	4	22.7	10.7	15-37	6-16
	Cardiac muscle	4	17.0	8.7	8-22	8-10
	Uterus	4	18.5	7.0	15-22	6-8
	Ovary	4	15.5	7.0	12-16	4-10
	Testicle	4	16.3	12.7	12-19	10-16
	Brain cortex	7	19.7	3.8	8-29	2-6
	Blood clot	6	20.5	4.2	8-30	3-6
	Plasma clot	10	18.2	5.2	15-25	2-6
	Cartilage	8	4.5	5.0	2-8	4-6
Rat	Thyroid	19	16.9	6.6	11-26	4-9
	Kidney	4	12.5	7.0	11-14	5-9
	Uterus	4	14.0	8.2	10-14	7-11
	Ovary	4	14.9	9.0	11-18	7-11
	Adrenal cortex	4	12.8	9.6	9-11	10-16
	Lymph node	4	14.8	10.0	10-20	9-11
	Lung	4	14.0	5.3	8-20	2-11
	Brain cortex	4	16.3	3.3	13-21	2-4
	Blood clot	4	16.3	3.3	11-21	2-4
	Decapsulated crushed lens	12	17.9	8.0	11-21	2-4
	Whole lens	8	6.3	8.0	5-11	2-14
	Crushed lens with capsule	4	16.5	7.5	12-20	5-10
	Tendon	4	7.1	10.3	5-8	7-14
	Cartilage	6	5.5	4.5	0-7	1-6
Pigeon	Thyroid	16	13.5	8.9	11-23	7-12
	Skin	6	9.3	12.6	7-12	8-12
Chicken	Thyroid	5	9.2	10.4	8-13	8-12

* The total number of experiments was 253. Included in this table are 14 experiments in which the counts were typical but in which the animals died before the experiments were concluded.

in lymphocytes as determined by means of differential counts occurs. Furthermore, in both the differential and the total counts the increase over the figures for the normal controls is approximately 16 per cent. If, as is shown in table 7, the absolute change in lymphocytes is calculated, the increase which takes place after homotransplantation is still more striking. There is an absolute average increase of 77.6 per cent in the case of the guinea pig and of 54.2 per cent in the case of the rat.

If all the tissues are classified according to the average time at which the maximum increase occurred, definite groups are found to exist. The earliest changes appear after homotransplantation of blood clot, plasma clot, brain cortex and lung, the maximum counts with these tissues being generally seen between the third and fifth day. However, increases in lymphocytes can often be noticed as early as the first or second day. The next group consists of homografts of thyroid, liver, kidney, uterus and crushed lens of the eye; here the maximum effect occurs usually between the sixth and eighth day. The ovary of the guinea pig also belongs to this group, while that of the rat belongs apparently to the next group, which consists of adrenal gland, lymph node, heart and skeletal muscle; with these organs the maximum increase is not reached until some time between the eighth and tenth day. In experiments with testicle and skin the maximum change

TABLE 7.—*Effect of Homotransplantation on the Distribution of Leukocytes in the Circulating Blood (Absolute Counts of Lymphocytes)*

Animal Species	Tissue	Experiments	Type of Count	Average Control Count	Average Maximum Count
Guinea pig	Thyroid	6	Total	7,775 leukocytes per cu. mm.	9,983 leukocytes per cu. mm.
			Differential	51.1% lymphocytes	70.7% lymphocytes
			Absolute	3,973 lymphocytes per cu. mm.	7,058 lymphocytes per cu. mm.
	7,058 — 3,973 = 3,085			3,085 / 3,973 × 100 = 77.8% absolute increase in lymphocytes	
Rat	Thyroid	6	Total	9,083 leukocytes per cu. mm.	11,058 leukocytes per cu. mm.
			Differential	58.5% lymphocytes	74.1% lymphocytes
			Absolute	5,314 lymphocytes per cu. mm.	8,194 lymphocytes per cu. mm.
	8,194 — 5,315 = 2,880			2,880 / 5,314 × 100 = 54.2% absolute increase in lymphocytes	

occurred even later, usually not before the twelfth day. There are, in addition, tissues which at no time following homotransplantation elicit the typical reaction. These include cartilage, tendon and intact lens of the eye. Transplantation of these yields results comparable to a certain extent, but perhaps not completely, to those obtained after autotransplantation or after merely operative interferences.

These variations in the time of the maximum reaction can be correlated with differences in the consistency of the various tissues, which apparently determine the readiness with which the effective substance is released from the grafts and reaches the hemopoietic organs in the host. In the first group, the tissues (blood clot, plasma clot and brain cortex) are very soft and loose, and the release is therefore greatly facilitated. On the other extreme are tissues such as cartilage, tendon, lens capsule and to a certain extent epidermis; these are hard tissues, which tend to prevent diffusion of the active substance. In addition, one has to consider the possibility that in these firmer tissues the

metabolism is low and that correspondingly the individuality differentials may be produced in only small amounts. In the case of cartilage the lack of a homoreaction has been confirmed by total counts. Intermediate between these two types of tissues are the glandular and muscular tissues, in which the maximum release of active substances takes place later than in the first group but with which the maximum counts are very high. The maximum reaction is reached somewhat more rapidly with glandular tissues than with muscle. The difference between ovaries of the rat and guinea pig may perhaps be due to the fact that in the ovary of the rat the corpora lutea are much more numerous than in the ovary of the guinea pig.

If one considers only the relative counts of lymphocytes, the degree of maximum increase of these cells shows no apparent correlation with the time at which this increase is reached. But an analysis of the absolute counts (table 6) shows that in those instances in which the maximum increase appeared on the fifth or sixth day after homotransplantation the increase was greater than in those in which the maximum increase was reached later, namely, on the eighth or ninth day; in the former the ascent of the curve representing this increase in the absolute number of lymphocytes was steeper than in the latter cases.

The fact that it was possible to observe typical generalized homoreactions following transplantation of homogenous blood clots or plasma clots is of particular interest since Loeb [14] could find only slight differences histologically between the reactions against autogenous and homogenous blood clots. The differences between the local and general reactions against blood clots are presumably due to the great rapidity with which the individuality differentials are extracted from this material, as is indicated by the very early appearance of the increase in lymphocytes in the general circulation; under this condition the local reaction has not had a chance to become manifest in full strength. The response on the part of the leukocytes in the circulation appears thus to be a very sensitive test for the action of a homotoxin. Furthermore, since the typical lymphocytic reaction in the peripheral blood occurred also after homotransplantation of plasma clot, constituents of the blood other than the red cells may also contain the organismal differentials.

The occurrence of a typical peripheral blood response to homotransplantation of crushed ocular lens is of particular interest. Uhlenhuth [15] showed on the basis of immunologic tests that the protein

14. Loeb.[2e] Loeb.[2p]

15. Uhlenhuth, P., in Festschrift zum sechzigsten Geburtstage von Robert Koch, Jena, Gustav Fischer, 1903, p. 49.

of the lens of the eye is organ specific but lacks species specificity almost entirely. The findings of Uhlenhuth have been confirmed and further extended by Hektoen and Schulhof.[16] Fleisher[17] used transplantation as a test for the presence of the individuality differential in this tissue. He was unable to observe any marked difference between the local lymphocytic reactions around autotransplanted and homotransplanted lens and concluded, therefore, that this tissue did not seem to possess an individuality differential. In accordance with a suggestion made by Loeb, Fleisher considered the possibility that the capsule of the lens presented a mechanical barrier to the interchange of substances between host and transplant and to the entrance of lymphocytes and fibroblasts of the host into the homogenous transplant, but he dismissed this possibility because the reaction around the lens was apparently lacking even in cases in which the capsule had been accidentally broken. My experiments with whole lens were likewise negative. On the other hand, crushed lens, whether previously decapsulated or not, always gave a typical homoreaction. Similarly in a case in which the whole lens was accidentally crushed during the process of transplantation a positive result was obtained. Thus, from my experiments it would seem that Loeb's explanation is the correct one. It is possible that in Fleisher's experiments the injury to the capsule was not sufficient to offer enough surface contact between lens fibers and host tissue or that the absorption of the active substance was too rapid to make possible a local reaction.

These experiments show, therefore, that the individuality differential is present in a wide variety of tissues of the body, a fact already well established by Loeb,[18] and that when these tissues are homotransplanted there takes place a typical general reaction, characterized by both an absolute and a relative increase in the number of lymphocytes in the peripheral blood, which corresponds to the local reaction about the transplanted tissue. This general reaction in the presence of homogenous tissue occurs only when a threshold quantity of homotoxin is released, a lesser amount eliciting merely a wound reaction. On the other hand, it seems that a greater than threshold quantity does not increase the general reaction to a noticeable extent. This was demonstrated by the fact that 6 guinea pigs in which multiple homotransplantations of thyroid gland were carried out simultaneously—2 animals receiving two, 2 others four and the last 2 six thyroid grafts—did not show a greater percental increase of lymphocytes than was observed following a single homogenous transplantation of thyroid, nor did the

16. Hektoen, L., and Schulhof, K.: J. Infect. Dis. **34**:433, 1924.
17. Fleisher, M. S.: J. M. Research **42**: (*a*) 173 and (*b*) 491, 1921.
18. Loeb.[2d] Loeb.[2q]

reaction appear earlier under these circumstances. It seems that the reaction requires a certain threshold amount of tissue; if this is exceeded the reaction is neither intensified nor accelerated.

Syngenesiotransplants.—Fourteen experiments were carried out in which thyroids were syngenesiotransplanted in guinea pigs, the tissues being transferred to brother or sister. The average maximum percental increase of lymphocytes was 11.7, distinctly less than that observed after homotransplantation. The variations in the maximum increase of lymphocytes ranged between 8 and 18 per cent. In 4 of these 14 experiments the increase was less than 10 per cent, while in only 2 it was above 15 per cent. Not only was the average maximum increase in the number of lymphocytes less after syngenesiotransplantation than after homotransplantation, but also the average time at which this maximum increase was reached was greater, namely, twelve and one-tenth days as compared with seven and one-tenth days in the corresponding homotransplantations. The time at which the maximum

TABLE 8.—*Effect of Syngenesiotransplantation on the Distribution of Leukocytes in the Circulating Blood (Differential Counts)*

Animal Species	Tissue Transplanted	Experiments	Average Maximum Percental Increase of Lymphocytes	Average Day of Maximum Increase	Range of Variation in Maximum Percental Increase	Range of Variation in Day of Maximum Increase
Guinea pig	Thyroid	14	11.7	12.1	8-18	6-19

increase occurred varied between six and nineteen days. Furthermore, in the 4 cases in which a maximum increase in lymphocytes of less than 10 per cent was shown the increase was reached between the sixth and nineteenth day after transplantation, whereas in the remaining 10 cases variations in time ranging from the sixth to the thirteenth day were exhibited. Thus some of the syngenesiotransplants elicited reactions similar to those observed after homotransplantation, while the reactions to others closely resembled those following autotransplantation. The largest group, consisting of 8 cases in which the maximum increase was somewhat less than that observed after homotransplantation and in which it usually appeared at a later time, behaved in an intermediate manner.

One can thus observe following syngenesiotransplantation reactions of intensities ranging from that observed after autotransplantation, at one extreme, to that seen following homotransplantation, at the other, with the greatest number of experiments giving intermediate responses. There are quantitative differences between the reactions observed after homotransplantation and those following syngenesiotransplantation. In

the latter a longer time is generally necessary for the release of a quantity of the strange organismal differentials sufficient to induce a general reaction.

Heterotransplants.—As shown in table 9, altogether 107 transplantations of a variety of heterogenous tissues were carried out. The most striking difference between the results in these experiments and those observed following homotransplantation consists in the kind of white blood cells which react. Whereas after homotransplantation there is both a relative and an absolute increase in the lymphocytes of the peripheral blood, grafting of heterogenous tissues is followed by a primary increase in the number of polymorphonuclear leukocytes and, again, this increase is both a relative and an absolute one.

Furthermore, the reaction to heterogenous grafts tends to appear a few days earlier than does the reaction to homogenous transplants (table 9). Following heterotransplantation of thyroid the increase in polymorphonuclear leukocytes reaches a maximum on about the fifth day; this is approximately two days earlier than that observed after homotransplantation. The beginning of the increase can be noticed already on the second or third day after heterotransplantation of thyroid; this corresponds to the time when the increase of lymphocytes becomes noticeable in the cases in which the easily absorbed blood clot is homotransplanted. This increase in polymorphonuclear leukocytes is followed by a regression which continues until approximately the normal count is attained, usually about the tenth day. Subsequently there takes place a secondary reaction characterized by an increase, both relative and absolute, in the number of lymphocytes, which usually reaches a maximum between the fourteenth and sixteenth days and again is followed by a regression to normal.

As was observed in the case of homotransplantation, there are differences in the time of reaction which depend on the kinds of tissue used for heterotransplantation. Here, too, blood clot and brain give the earliest reactions, the glandular tissues intermediate ones, and heterotransplanted cartilage and tendon, which after homotransplantation gave no reaction at all, produce a definite change characterized by a relatively late appearance of the maximum increase, usually about the seventh day. Crushed lens also belongs to the category of tissues giving a tardy reaction.

Likewise, after heterotransplantation of plasma clot a typical reaction is obtained; as after homotransplantation, it is later in its appearance than that following heterotransplantation of whole blood (table 9). It thus seems that the substances acting as, or given origin to, homotoxins and heterotoxins are present in the red blood corpuscles as well as in the fibrin, but the latter contains an amount sufficient only to cause a retarded reaction.

Furthermore, tissues such as adrenal, and in the rat also ovary, which called forth fairly late maximal responses after homotransplantation react similarly following heterotransplantation, while testicle, which caused a relatively late change when homografted, induces a heteroreaction within the same period of time as do glandular tissues such as thyroid, liver and kidney. It should also be noted that crushed lens is capable of producing a typical heteroresponse and contains, therefore, heterodifferentials as well as homodifferentials.

TABLE 9.—*Effect of Heterotransplantation on the Distribution of Leukocytes in the Circulating Blood (Differential Counts)*

Animal Species	Tissue Transplanted	Experiments*	Average Maximum Percental Increase of Polymorphonuclears	Average Day of Maximum Increase	Range of Variation in Percental Maximum Increase	Range of Variation in Day of Maximum Increase
Pigeon to guinea pig	Thyroid	9	16.6	5.6	13-23	5-6
	Cartilage	4	16.0	6.0	12-23	5-6
	Blood clot	4	26.7	3.5	23-34	2-5
Guinea pig to pigeon	Thyroid and cartilage	4	18.8	5.0	15-23	4-6
Rat to guinea pig	Thyroid	10	19.2	4.9	12-29	3-10
	Cartilage	6	13.8	6.8	11-16	6-8
	Blood clot	6	19.5	3.5	12-28	1-5
Guinea pig to rat	Thyroid	4	16.8	5.0	8-23	4-6
	Uterus	4	13.0	5.0	10-16	4-6
	Ovary	4	18.5	9.0	14-25	8-10
	Adrenal cortex	4	17.5	7.0	14-20	6-8
	Testicle	4	12.0	5.5	9-14	2-8
	Brain cortex	4	18.0	4.5	11-24	2-8
	Blood clot	4	15.5	3.5	10-23	2-5
	Decapsulated crushed lens	8	20.6	7.3	12-32	4-10
	Tendon	4	13.3	4.5	12-15	2-6
	Lymph node	4	14.5	4.5	9-20	4-6
Mouse to rat	Cartilage	4	12.3	5.0	9-17	4-6
	Liver	4	14.3	5.0	12-17	4-8
	Kidney	4	20.0	5.0	13-29	4-8
Rabbit to guinea pig	Plasma clot	4	15.3	5.0	9-19	4-8
Rabbit to rat	Plasma clot	4	22.0	7.5	18-25	6-8

* The total number of experiments was 107.

The results of total counts as determined in a series of 8 experiments parallel those obtained by means of differential counts. However, when the absolute increase in the number of polymorphonuclear leukocytes is determined from these data, it is seen that this increase is much greater than that indicated by the differential counts, since the magnitude of change exceeds 100 per cent. Furthermore, the absolute increase of polymorphonuclear leukocytes which takes place in response to heterotransplantation is much greater than the absolute increase of lymphocytes in the case of homotransplantation. The reaction following heterotransplantation is therefore more acute and severe than that following homotransplantation. In the former it is the

polymorphonuclear leukocytes which react rather than the lymphocytes; the rapidity with which the reaction takes place is greater, and the magnitude of the increase exceeds that occurring after homotransplantation.

The secondary increase in lymphocytes previously mentioned, which occurs between the tenth and fourteenth days, parallels closely, as far as magnitude is concerned, the primary increase following homotransplantation. In the 4 experiments in which the absolute increase in polymorphonuclear leukocytes after transplantation of rat tissues into guinea pigs had been determined, the secondary average maximum increase in lymphocytes amounted to 14.5 per cent; it occurred, on the average, fourteen and six-tenth days after the operation.

Finally experiments were carried out in which pieces of mouse carcinoma which had been serially propagated by means of homografts

TABLE 10.—*Effect of Heterotransplantation on the Absolute Counts of Polymorphonuclear Leukocytes in the Circulating Blood*

Animal Species	Tissue Transplanted	Experiments	Type of Count	Average Control Count	Average Maximum Count
Rat to guinea pig	Thyroid	4	Total	6,988 leukocytes per cu. mm.	9,050 leukocytes per cu. mm.
			Differential	32.0% polymorphonuclears	51.8% polymorphonuclears
			Absolute	2,236 polymorphonuclears per cu. mm.	4,688 polymorphonuclears per cu. mm.
	4,688 − 2,236 = 2,452			2,452 / 2,236 × 100 = 109.6% absolute increase in polymorphonuclear leukocytes	

were transplanted into 4 rats and 4 guinea pigs. The differential counts showed a typical heteroreaction in each case consisting in an early increase in lymphocytes. Five of the animals exhibited the maximum secondary lymphocytic reaction between the seventh and tenth days, while the remaining ones showed typical secondary reactions between the tenth and fourteenth days.

EFFECT OF SUCCESSIVE TRANSPLANTATIONS ON THE DISTRIBUTION OF LEUKOCYTES IN THE PERIPHERAL BLOOD

Successive transplantations of pieces of tumors into the same host and other experiments of a related nature have shown that by this procedure the resistance of the host against the grafted tumor can be increased. According to Lumsden [19] and Woglom,[20] immune substances can be demonstrated in the blood serum of animals actively immunized against a tumor or in which an implanted tumor retrogresses apparently

19. Lumsden, T.: Am. J. Cancer **15**:563, 1931.
20. Woglom, W. H.: Cancer Rev. **4**:129, 1929; Am. J. M. Sc. **181**:157, 1931.

spontaneously. Furthermore, Schoene [21] carried out a series of experiments in which he attempted to demonstrate that immunity developed against a graft of normal tissue (skin) as a result of a preceding injection of a suspension of liver, spleen or kidney. In his experiments the tissue used for immunization belonged to the same species as the subsequently grafted skin. In homotransplantation in rabbits he believed that he had succeeded in producing immunity; in mice, on the other hand, his attempts proved to be unsuccessful. Because of the many variable factors which enter into the success of a skin graft, and since in the large majority of cases a homogenous skin graft remains united with the host only temporarily, these results are not quite convincing as far as homotransplantations are concerned. In the case of successive heterotransplantations of mouse skin on rats, on the other hand, Schoene's results seem more convincing.

Loeb [2q] approached this problem by means of successive homotransplantations of the same type, as well as of other types, of tissue and could find no definite acceleration of the reaction against a second homotransplant as compared with that against a first graft. Fleisher [22a] also found that previous immunization of an animal by injection of suspensions of homogenous tissue did not alter the nature or the course of the reaction which took place around a homotransplant. However, in the case of heterotransplantation he [22b] did find an increased reaction on the part of polymorphonuclear leukocytes, which surrounded and invaded the graft in the first few days in an animal immunized against tissues of that particular species; in addition, he noticed a delay in the ingrowth of fibroblasts and interference with the slight growth processes which may take place in the heterogenous tissue.

In order to determine whether acceleration of the reaction following a second transplantation could be detected by means of blood counts, a series of 82 experiments was carried out in which two successive homotransplantations were made, and in 23 additional experiments the effects of two successive heterotransplantations on the number of leukocytes were determined. In the case of the homotransplantations a first subseries of experiments consisted of 58 in which an interval of ten days intervened between the two transplantations while in a second subseries, consisting of 24 experiments, the length of the intervening period was twenty-one days. Twelve experiments in each group served as controls; in these, inert paraffin took the place of the first tissue graft. Rats as well as guinea pigs were used as hosts.

Successive Homotransplantations.—Homotransplantations of thyroid after a previous implantation of paraffin called forth a typical homo-

21. Schoene, G.: München. med. Wchnschr. **59**:457, 1912.
22. Fleisher, M. S.: (*a*) J. M. Research **38**:191, 1918; (*b*) **37**:483, 1918.

reaction as far as the magnitude of increase and the average time of maximum reaction were concerned. On the other hand, if the first transplant consisted of living homogenous tissue, the reaction following the subsequent transplantation of the same or of another kind of tissue of the same species was definitely accelerated; it occurred from two to four days earlier than the reaction after the first grafting. As is shown in table 11, there is no difference between the results obtained in the first subseries, in which the interval between the two transplantations was ten days, and the results obtained in the second one, in which the interval was twenty-one days.

Despite the acceleration of the reaction, the actual increase in the number of lymphocytes following the second transplantation was less than that following the first transplantation in all cases except 5 (noted in part 1 of table 11) in which the increase following the second grafting was greater than that observed after the first.

Three experiments in which four successive homotransplantations of blood clot were made into guinea pigs at four day intervals failed to reveal any diminution in the number of lymphocytes after the second, third or fourth transplantation nor was there any evidence of a more rapid appearance of the lymphocytic increase. This is probably at least partly due to the fact that the reaction to a first implantation of blood clot is already a very rapid one; the maximum increase occurs usually between the second and the fourth day.

In addition to the experiments shown in table 11 there was a series of 8 experiments—4 in guinea pigs and 4 in rats—in which not only differential counts but also total counts were made. The changes established by means of the latter closely paralleled the results of the differential counts. In the guinea pigs there was a maximum average increase of 1,585 leukocytes per cubic millimeter of peripheral blood following the primary transplantation, and in the rats, a corresponding average increase of 2,010 leukocytes. The increases following the second transplantations were on the average 1,150 leukocytes in the guinea pigs and 1,220 in the rats. The average period of time preceding the primary maximum increase was seven and one-tenth days in the guinea pigs and six and eight-tenth days in the rats, whereas the average period preceding the secondary maximum increase was four and five-tenths days in the former and four and three-tenths days in the latter.

Successive Heterotransplantations.—The reactions of the leukocytes to successive transplantations of heterogenous tissues are shown in table 12; they are quite comparable to those following successive transplantations of homogenous tissues except that the changes primarily involve the polymorphonuclear leukocytes instead of the lymphocytes.

With both kinds of successive transplantations the maximum average increase in leukocytes was less after the second than after the first transplantation, but this maximum reaction was reached at an earlier time. The reactions were not noticeably affected by a preceding implan-

TABLE 11.—*Changes in Counts of Lymphocytes Following Successive Homotransplantations*

Animal Species	Experiments*	Tissue Transplanted	Average Maximum Percental Increase of Lymphocytes	Average Day of Maximum Increase	Range of Variation in Percental Increase	Range of Variation in Day of Maximum Increase
	1. Interval of Ten Days Between First and Second Transplantation					
Guinea pig	8 control experiments	1—paraffin	4.8	1.5	0-5	4-10
		2—thyroid	16.4	6.0	8-27	4-8
	9	1—thyroid	21.0	7.3	13-28	6-8
		2—thyroid	18.7	3.6	11-29	2-4
	8	1—thyroid	17.0	7.9	10-29	8-10
		2—liver	14.3	4.0	9-25	2-4
	6	1—liver	14.3	7.3	10-20	4-10
		2—liver	13.7	4.0	11-25	2-4
	6	1—liver	12.7	6.3	10-16	5-7
		2—thyroid	11.3	4.0	8-19	2-4
	4	1—kidney	11.5	6.5	10-15	5-7
		2—thyroid	10.5	4.5	7-15	3-7
	4	1—kidney	14.5	8.5	11-18	7-10
		2—kidney	10.0	4.0	8-12	2-4
	4	1—liver	14.0	7.5	12-17	3-10
		2—kidney	11.5	5.0	8-17	2-10
	2	1—thyroid	17.5	6.0	14-21	Both 6
		2—kidney	14.5	4.0	14-15	Both 4
Rat	4 control experiments	1—paraffin	4.5	2.5	3-5	2-4
		2—thyroid	17.5	6.0	11-19	4-8
	1	1—thyroid	18.0	8.0		
		2—thyroid	11.0	4.0		
	2	1—thyroid	13.5	6.0	13-14	Both 6
		2—liver	11.0	4.0	9-13	Both 4
	2. Interval of Twenty-One Days Between First and Second Transplantation					
Guinea pig	8 control experiments	1—paraffin	4.0	7.5	3-6	1-16
		2—thyroid	11.0	6.9	2-21†	5-8
	8	1—thyroid	14.3	7.3	11-18	5-8
		2—thyroid	11.5‡	3.0	8-15	2-4
	4 control experiments	1—paraffin	4.3	8.3	3-6	1-12
		2—thyroid	15.5	6.5	11-22	5-8
	4	1—thyroid	17.0	8.0	11-17	6-9
		2—thyroid	12.3	4.0	9-15	2-5

* The total number of experiments in group 1 was 58; that in group 2 was 24.
† One animal failed to react to the thyroid transplant.
‡ One animal showed a greater increase after the second transplantation than after the first, but this increase appeared as early as on the second day after the second transplantation as compared with the maximum increase on the eighth day after the first transplantation.

tation of an inert foreign substance, such as paraffin. In addition to the differential counts, a number of total counts were also made in this series; these are shown in table 13. The total counts closely paralleled the differential counts.

Not only is the increase in polymorphonuclear leukocytes accelerated in the case of a second heterotransplantation, but the later phase of the

reaction which takes place after heterotransplantation, consisting of an increase in lymphocytes, is also accelerated. Instead of occurring between the tenth and fourteenth days it usually takes place between

TABLE 12.—*Changes in Differential Counts of Polymorphonuclear Leukocytes Following Successive Transplantations of Heterogenous Tissues*

Animal Species	Experiments*	Tissue Transplanted	Average Maximum Percental Increase of Polymorphonuclears	Average Day of Maximum Increase	Range of Variation in Percental Increase	Range of Variation in Day of Maximum Increase
Rat to guinea pig	4 control experiments	1—paraffin	(Typical reaction to an inert substance)			
		2—thyroid	20.5	5.3	12-30	4-6
	7	1—thyroid	25.2	5.8	12-29	4-10
		2—thyroid	13.4	3.0	9-34	2-4
	1	1—thyroid	21.0	6.0		
		2—kidney	12.0	4.0		
	6	1—cartilage	14.8	5.3	11-23	4-8
		2—thyroid	10.0†	3.3	7-16	2-4
	1	1—thyroid	18.0	6.0		
		2—liver	13.0	4.0		
Guinea pig to rat	2	1—thyroid	12.5	6.0	12-13	Both 6
		2—liver	8.5	4.0	5-12	Both 4
	1	1—thyroid	19.0	6.0		
		2—thyroid	12.0	4.0		
	1	1—thyroid	10.0	6.0		
		2—kidney	5.0	2.0		

* The total number of experiments was 23.
† In three cases an increase in the relative number of polymorphonuclear leukocytes of less than 10 per cent followed the second transplanation.

TABLE 13.—*Changes in Total Counts of Leukocytes Following Successive Transplantations of Heterogenous Tissues*

Animal Species	Experiments*	Tissue Transplanted	Average Maximum Increase of Leukocytes per Cu. Mm.	Average Day of Maximum Increase	Range of Variation in Maximum Increase	Range of Variation in Day of Maximum Increase
Rat to guinea pig	2	1—thyroid	1,950	6.0	1,550-2,350	Both 6
		2—thyroid	1,100	4.5	900-1,300	4-5
	1	1—thyroid	2,200	5.0		
		2—kidney	1,450	4.0		
	1	1—thyroid	1,750	6.0		
		2—liver	900	4.0		
Guinea pig to rat	2	1—thyroid	2,350	5.5	2,000-2,700	5-6
		2—liver	1,200	3.5	900-1,500	3-4
	1	1—thyroid	1,900	5.0		
		2—thyroid	950	4.0		
	1	1—thyroid	1,750	6.0		
		2—kidney	1,200	2.0		

* The total number of experiments was 8.

the seventh and tenth days. In all my experiments the second heterotransplantation was carried out ten days after the first grafting, at a time, therefore, when the increase in lymphocytes caused by the pri-

mary graft should have been just beginning. But this increase in lymphocytes caused by the primary graft was never actually observed, being apparently obliterated by the increase in polymorphonuclear leukocytes caused by the second transplant. The lymphocytic reaction which appeared under these conditions was approximately of the same magnitude as the corresponding reaction to a single heterotransplantation.

EFFECT OF INJECTIONS OF TRYPAN BLUE ON THE CHANGES IN LEUKOCYTE COUNTS FOLLOWING HOMOTRANSPLANTATION AND HETEROTRANSPLANTATION

Trypan blue was injected over different periods, two, four and six days, respectively, preceding homotransplantation as well as heterotransplantation and again at intervals of two and four days immediately after transplantation, as well as at weekly intervals thereafter. Differential counts were made during the period of the preliminary injections, as well as every other day for a period of twenty days after transplantation. Guinea pigs served as hosts. They were divided into two groups of 6 animals each. The first was given injections of a 0.5 per cent solution of trypan blue in 0.5 cc. amounts; the second group, the same solution in 1 cc. amounts. Three animals in each group received homotransplants of thyroid gland. Three received heterotransplants of rat thyroid.

The preliminary injections of trypan blue as such in either dosage for periods of two, four or six days did not markedly increase the percentage of lymphocytes or of polymorphonuclear leukocytes. In 3 guinea pigs the lymphocytes increased slightly above 5 per cent. One showed an increase of as much as 8 per cent on one occasion. Generally, however, the results observed were similar to those previously described in normal controls, in which slight percentage deviations in a positive as well as in a negative direction occurred.

As a result of the transplantation, the lymphocytes rose generally to about 5 per cent, an effect which could be attributed to the operative interferences, and it was identical with that observed after autotransplantation or the implantation of inert substances. In two other experiments variations in leukocyte counts similar to those occurring in normal controls were seen. The changes characteristic of homotransplantation and heterotransplantation were prevented by the injections of trypan blue.

There were, however, three exceptions to these results, and all of these occurred in the animals receiving the smaller doses of trypan blue; 2 of these guinea pigs had received homografts, while the third one was the host of a heterotransplant. Moreover, 2 of these 3 animals had

been given injections of trypan blue only during a two day period; the third one had been given injections during a four day period preceding transplantation. These animals showed the usual reactions characteristic of transplantation of homogenous or heterogenous tissue, although in all of them the maximum increase in the number of leukocytes occurred rather late, namely, on the ninth day following the homotransplantations, and on the eighth day in the animal which had received a heterotransplant, an indication that even in these cases the reaction had been somewhat weakened.

When the transplants were removed from the host animals at autopsy, it was observed that the majority of them were surrounded by a blue peripheral ring. Whether the deposit of the dye in the periphery of the transplant prevented the diffusion of the organismal differential into the host, or whether the trypan blue prevented the reaction on the part of the leukocytes through a blockade of the reticuloendothelial system must be left undecided for the present.

EFFECT OF HEAT ON ORGANISMAL DIFFERENTIALS

In earlier investigations on tumors (Loeb [2c]) and on amebocytes (Loeb and Drake [22a]) it was shown that by exposure of tissues or cells to graded degrees of heat it was possible to induce a reduction of abnormalities in vital processes without causing actual death in the exposed cells and that under these conditions recovery of the impaired tissues might take place. Siebert [23] exposed pieces of normal tissues in vitro to higher temperatures before transplantation. Heating homogenous thyroid and cartilage at temperatures varying between 43 and 51 C. for thirty minutes caused a marked diminution in the strength of the lymphocytic reaction toward the homogenous graft, but it caused only a slight reduction in the reaction of the polymorphonuclear leukocytes and lymphocytes toward heterotransplants.

I studied the effect of heating tissues in vitro preceding transplantation on the subsequent action which homotransplants and heterotransplants exerted on the number and on the kinds of leukocytes in the circulating blood.

Various tissues were excised and subjected outside the body to different degrees of heat. After the tissue was removed from the animal, it was immersed in a sterile solution of 0.9 per cent sodium chloride which filled part of a test tube. The latter, containing the piece of tissue, was then placed for one-half hour in a water bath, previously adjusted to the desired temperature, which was kept constant. At the end of the period of heating, the tissue was removed from the test tube and either homotransplanted or heterotransplanted. Differential counts were made in the usual manner.

22a. Loeb, L., and Drake, D.: J. M. Research **44**:447, 1924.

23. Siebert, W. J.: Arch. Path. **12**:590, 1913.

Altogether, 69 experiments of this kind were carried out with homotransplanted and heterotransplanted tissues (table 14). The reactions were classified as typical if both the magnitude and the time of the maximum increase were within the limits established for the transplanted normal tissues. If the magnitude of the increase was about the same but the time of appearance later than normal, the reaction was designated as delayed. If the time of appearance was late but the maximum increase was only from 5 to 10 per cent, the reaction was termed weak, whereas if the increase was 5 per cent or less, the reaction was considered negative.

Effect on the Individuality Differential.—As can be seen from table 14, thyroid heated at 45 or 50 C. and then homotransplanted calls forth a normal or about normal reaction. There were 18 typical reactions, 2 were delayed, and in only a single experiment, that of homotransplantation of thyroid of the rat, did the guinea pig fail to show a reaction. Homotransplants of pigeon skin previously exposed to these two temperatures gave 5 typical reactions and 4 weak ones. However, the fact may be recalled that even normal, nonheated skin gives only a weak reaction after homotransplantation. These experiments suggest that heating at 45 or 50 C. may enhance the reaction elicited by skin to some extent, since the number of typical reactions observed under these conditions was greater than that following homotransplantation of the normal tissue. But further experiments will be necessary to make such a conclusion definite.

After being heated at 52 C., thyroid of the rat when homotransplanted called forth a typical reaction. Pigeon thyroid heated at 54 C. previous to homotransplantation induced typical reactions in 3 experiments, a weak reaction in 1 and no reaction in 2. Pigeon skin and thyroid and rat thyroid heated at 56 C. were inactive. The critical temperature in the case of homografted thyroid in pigeon seems thus to be approximately 54 C. Rat thyroid was not exposed to a temperature of 54 C., but since this tissue after being heated at 52 C. called forth normal reactions, whereas tissue previously heated at 56 C. failed to do so, it is probable that the critical temperatures for pigeon and rat thyroids are similar and that these tissues are inactivated at about 54 C. or at a slightly higher temperature.

Heating at 56 C. destroyed the action of guinea pig thyroid. However, guinea pig kidney heated at 56 C. for one-half hour and subsequently homotransplanted gave positive reactions in 2 of 4 cases; in 1 case the reaction was weak and in the fourth it was negative. These results indicate that in kidney tissue the homodifferential may be somewhat better protected against the injurious action of heat than in thyroid tissue.

In 8 experiments homotransplantation of pigeon thyroid was preceded by successive freezing and thawing. The thyroid gland was removed from a donor animal and immersed in sterile 0.9 per cent sodium

chloride solution in a test tube which was kept in an ice and salt mixture at 0 C. for one-half hour. The test tube containing the tissue was then kept at room temperature likewise for thirty minutes, when thawing took place. This process was repeated three times. Following the last thawing the tissue was homotransplanted. As can be seen in table 14, in 5 cases typical reactions were elicited, in 2 cases the reactions were delayed, and in 1 instance the reaction was weak. No negative reactions were observed. It thus appears that repeated freezing and thawing did not destroy the individuality differential but injured it to a slight extent.

Effect on the Species Differential.—Similar experiments were carried out with heterogenous tissues, but the latter were exposed to somewhat higher temperatures than homogenous tissues. Rat thyroid heated at 56 C. for one-half hour failed to give a reaction. The same negative result was obtained when guinea pig thyroid previously heated at 56 C. was transplanted to the rat. On the other hand, both kidney and cartilage, after having been heated at the same temperature, elicited the typical heteroreactions. Transplantation from rat to guinea pig of kidney previously heated at 60 C. for one-half hour gave 1 positive and 3 negative reactions, whereas in the reciprocal experiments no positive reactions were obtained. But with cartilage heated at 60 C. 50 per cent positive and 50 per cent negative reactions were noted; this, therefore, seems to be close to the critical temperature for this tissue, since after being heated at 65 C. cartilage failed to elicit a response.

From these experiments it may be concluded that the organismal differentials are destroyed at temperatures ranging approximately between 54 and 60 C., and furthermore, that the organismal differentials in denser tissues, such as cartilage, are somewhat more resistant to the injurious effect of heat than those in softer tissues, such as thyroid. Kidney stands midway between these two kinds of tissues as far as both density of structure and resistance to the injurious effects of heating are concerned. It needs still to be determined whether extraction of the organismal differentials occurs in these experiments and complicates their interpretation.

EFFECT OF TREATMENT OF TISSUES WITH CHEMICAL SUBSTANCES ON THE ORGANISMAL DIFFERENTIALS

In addition to studying the effect of heat on the organismal differentials I have begun an investigation of the action of various chemical substances on the homodifferential. So far it may be stated that solutions of 0.9 per cent sodium chloride in distilled water to which a few small crystals of thymol have been added in order to prevent the growth of bacteria seem to be relatively innocuous; but the large majority of the substances used proved very injurious, especially those which tended

to denature proteins, for such substances abolished the reactions to homodifferentials in the peripheral blood.

EFFECT OF PARENTERAL ADMINISTRATION OF PROTEINS, CARBOHYDRATES AND LIPOIDS ON THE DISTRIBUTION OF LEUKOCYTES IN PERIPHERAL BLOOD

As controls for the effects exerted by the different kinds of tissue transplants on the numbers of leukocytes circulating in the peripheral blood, a series of experiments was carried out in which different proteins,

TABLE 14.—*Effects of Heat on Organismal Differentials*

Animal Species	Tissue	Temperature, C.	Reaction			
			Typical	Delayed	Weak	Negative
	1. Effect of Heat on the Individuality Differential *					
Pigeon	Thyroid	0	5	2	1	..
Pigeon	Thyroid	45	6	..	..	..
Pigeon	Skin	45	3	..	2	..
Rat	Thyroid	45	3	1	..	..
Pigeon	Thyroid	50	4	1	..	..
Pigeon	Skin	50	2	..	2	..
Rat	Thyroid	50	5	..	..	1
Rat	Thyroid	52	4	..	..	..
Pigeon	Thyroid	54	3	..	1	2
Pigeon	Thyroid	56	..	..	..	4
Pigeon	Skin	56	..	..	..	4
Rat	Thyroid	56	..	..	..	6
Guinea pig	Thyroid	56	..	..	..	3
Guinea pig	Kidney	56	2	..	1	1
	2. Effect of Heat on the Species Differential †					
Rat to guinea pig	Cartilage	56	6	..	..	..
	Thyroid	56	..	..	..	3
Guinea pig to rat	Kidney	56	6	..	..	..
	Cartilage	56	4	..	..	..
	Thyroid	56	..	..	..	4
Rat to guinea pig	Kidney	60	1	..	..	3
	Cartilage	60	2	..	2	..
	Thyroid	60	..	..	..	2
Guinea pig to rat	Kidney	60	..	..	..	4
	Cartilage	60	2	..	..	2
	Thyroid	60	..	..	..	4
	Cartilage	65	..	..	..	6

* The total number of experiments was 69.
† The total number of experiments was 51.

carbohydrates and fatty substances were parenterally administered to guinea pigs and rats (table 15). These substances, whenever feasible, were implanted in a solid or semisolid state in order to reproduce as much as possible the mode of procedure used in the preceding experiments. In other cases liquid substances were injected. A plus in the table represents a positive reaction, which consisted in an increase in lymphocytes exceeding 12 per cent, occurring generally between the second and fourth days after the initial administration of the substance. This was the only positive response to the administration of certain of

these substances which took place, as will be discussed later. It corresponds to the reaction which is characteristic of homogenous tissues, as well as of homogenous blood and plasma clots, as far as the nature and magnitude of the reaction are concerned; as for the time at which the maximum of the increase in lymphocytes was reached, the reaction corresponds to that following homotransplantation of blood and plasma clots and brain, substances which are relatively soft, but not to that following homotransplantation of the large majority of tissues. However, the reaction differs in other important respects from that elicited by homogenous tissues.

Only the proteins were observed to induce this type of reaction. Gelatin implanted in the form of flakes had this effect. On the other hand, in the case of a single injection of a readily absorbable protein, such as fresh egg albumin, the reaction did not follow, owing, no doubt, to the great rapidity with which this substance is eliminated. When, however, egg albumin was injected on three successive days, the protein was retained sufficiently long to call forth the typical response on the part of the lymphocytes.

It is of special interest that neither partial denaturation of the albumin, caused by an addition of thymol crystals, nor complete denaturation, produced by an exposure to the temperature of boiling water, was followed by a loss of the ability of the protein to induce this reaction.

Casein, which is of animal origin, and edestin, a plant derivative, acted similarly to egg albumin.

These findings suggested tests with heterogenous blood serum. For this purpose use was made of rabbit serum, which was injected subcutaneously into rats or, after concentration, was implanted subcutaneously. Rabbit serum likewise did not induce a heterogenous reaction but a reaction similar to that seen after transplantation of homogenous tissues. There was a maximum increase in the number of lymphocytes, ranging from 15 to 25 per cent in the different experiments; the time of maximum reaction varied between the second and the fifth day. It may therefore be concluded that a mixture of rabbit serum albumin and globulin behaves in the same way as egg albumin and that it differs markedly in its effects from plasma clot, which acts like normal tissues. This difference in behavior between plasma clots and the corresponding blood serum is presumably due to the presence of fibrinogen in the former substance.

Of interest also is the fact that pieces of mouse embryo that had developed to a length of approximately 15 mm. did not, after transplantation in the rat elicit a heterogenous but a lymphocytic reaction characteristic of homogenous tissues; it is probable that the organismal differentials are not yet fully developed in these embryonal cells.

In addition to the differential counts of leukocytes, total counts were made on the peripheral blood of guinea pigs into which casein and edestin had been implanted. There was not only a relative increase in the number of lymphocytes but there was also an absolute increase comparable to the increase found in the differential counts following the administration of these substances. The absolute increase calculated from these data was also similar to that observed following homotransplantation, since in the 6 guinea pigs observed, 3 of which received casein and 3 edestin, this increase ranged between 58 and 81 per cent and averaged 69 per cent.

TABLE 15.—*Effect of Parenteral Administration of Proteins, Carbohydrates and Lipoids on the Distribution of Leukocytes in the Peripheral Blood*

Substances	Animal Species	Method of Administration	Experiments	Reaction
Gelatin	Guinea pig	Implanted flakes	4	+
Fresh egg albumin	Guinea pig	Single injection	10	—
Fresh egg albumin	Guinea pig	3 successive injections	4	+
Fresh egg albumin and thymol	Guinea pig	Single injection	6	+
Casein	Guinea pig	Implant paste	3	+
Edestin	Guinea pig	Implant paste	3	+
Fresh egg albumin	Rat	Single injection	4	—
Fresh egg albumin	Rat	3 successive injections	3	+
Fresh egg albumin and thymol	Rat	Single injection	4	+
Heat coagulated albumin	Rat	Implant	4	+
Casein	Rat	Implant paste	4	+
Edestin	Rat	Implant paste	4	+
Sodium oleate	Rat	Implant	4	—
Sodium stearate	Rat	3 successive injections	4	—
Glycogen	Rat	3 successive injections	4	—
Saccharose	Rat	3 successive injections	4	—
Starch	Rat	Implant paste	4	—
Lecithin	Rat	Implant	4	—
Olive oil	Rat	3 successive injections	4	—
Normal rabbit serum	Rat	3 successive injections	4	+
Concentrated rabbit serum	Rat	Implant	4	+
Mouse embryo	Rat	Implant	4	+

All other substances used, carbohydrates as well as fatty substances, failed to induce a specific reaction; they induced merely the change characteristic of the operative procedures.

While the effect exerted by proteins on the number and kinds of leukocytes in the circulating blood is apparently the same as that elicited by homogenous tissue transplants, it differs from the latter in two important respects: (1) it is not prevented by exposure to a degree of heat which would completely destroy the action of all tissue, and (2) it is produced by material of heterogenous origin. It is therefore probable that one has to deal with a nonspecific or rather with a less specific reaction, characteristic of various proteins, which under the conditions in which they were administered in our experiments elicited only a mild disturbance in the organism, while in the majority of experiments of earlier investigators the strange proteins were injected intravenously

and under these conditions induced acute changes, becoming noticeable usually in the course of the first day after the injection. Schittenhelm and his collaborators [24] and Wallbach [25] observed, immediately following a single injection of such a substance, leukopenia, which in some of Wallbach's experiments lasted for only a few minutes; this was followed by leukocytosis, which had a duration of about six hours, after which the count returned to normal. A subsequent injection of the same material produced an anaphylactic reaction in the animal and, in addition, prolonged both phases, the leukopenia as well as the leukocytosis; under this condition the leukocytosis could be extended over a period of four to six days. Wallbach was able to produce such reactions with whole serum, but he observed that various fractions of the serum containing isolated proteins, such as albumin and globulin, were ineffective. The injection of bacteria was also followed by a first phase of leukopenia and a second phase of leukocytosis; Wallbach interpreted the prolongation of the leukopenic reaction as indicative of a lack of development of an active immunity to the injected micro-organisms.

On the other hand, Wiseman [26] reported experiments in which embryonic chick extract, egg albumin or normal horse serum was repeatedly injected into animals either subcutaneously or intravenously. He found an increase in the total number of lymphocytes in the peripheral blood, varying from 23 to 139 per cent, which was noticeable from the seventh to the ninth day, in contrast to the reaction described by the aforementioned investigators, in which the leukocytes appeared on the first day. Furthermore, Wiseman's article contains no record of an abrupt rise in the animal's temperature accompanying the changes in the peripheral blood such as was noticed in the experiments of the other investigators.

It appears, therefore, that the changes which I have recorded are somewhat comparable to those observed by Wiseman but are different from those reported by Schittenhelm and Weichardt and also by Wallbach. The reactions which the latter workers observed were of a very acute nature, caused by intravenous injection of foreign proteins, a procedure which in a number of instances led to the death of the animal. The reactions of Wiseman and those which I have reported here, on the other hand, are of a milder character and due to a less active though long-lasting stimulation of the hemopoietic tissues.

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24. Schittenhelm, A.; Weichardt, W., and Grisshamer, W.: Ztschr. f. exper. Path. u. Therap. **10**:412, 1912. Schittenhelm, A., and Ströbel, H.: ibid. **11**:102 and 108, 1912. Schittenhelm, A., and Weichardt, W.: ibid. **14**:609, 1912.

25. Wallbach, G.: Ztschr. f. d. ges. exper. Med. **82**:22, 1932; Folia haemat. **58**: 393, 1937.

26. Wiseman, B. K.: J. Exper. Med. **53**:599, 1931.

COMMENT

In the various chapters of the preceding report I have already discussed the principal results of these investigations. I shall now discuss the main conclusion which can be drawn from the experiments, and I shall try to correlate the data with those previously obtained by Loeb and his collaborators [2] in their study of the effects of organismal differentials on the local reaction in and around grafts.

The evidence presented in this paper proves that a generalized reaction characterized by both an absolute and a relative increase in lymphocytes in the peripheral blood follows homotransplantation and syngenesiotransplantation and that a corresponding increase in polymorphonuclear leukocytes follows heterotransplantation. These changes take place in addition to the localized changes in and around the transplant. Furthermore, in its main outlines the leukocyte response closely parallels the types of changes taking place locally. Thus, following homotransplantation and syngenesiotransplantation it is the lymphocytes which collect around the graft and then invade it, whereas the cellular changes about heterotransplanted tissue in the early stages consist largely in an accumulation of polymorphonuclear leukocytes.

Moreover, the two reactions, the general and the local one, coincide as far as time factors are concerned. Thus after homotransplantation of thyroid the lymphocytes begin the invasion of the transplant by the sixth or the seventh day, the approximate time at which the maximum increase in lymphocytes is observed in the peripheral blood. After heterotransplantation the changes described by Loeb [2r] are of a more acute nature, leading to rapid destruction of tissue and involving, in the early stages, the polymorphonuclear cells rather than the lymphocytes. Correspondingly, here also the blood changes indicate a more acute reaction; the polymorphonuclear leukocytes are primarily increased, and they appear at an even earlier date than do the lymphocytes in the case of syngenesiotransplantation and homotransplantation, the maximum increase being reached on the fourth or the fifth day in the case of thyroid heterografts as compared with the maximum increase on the sixth or the seventh day following homotransplantation of the same kind of tissue.

The degree of increase of the leukocytes is also greater following heterografting than after homografting. In the case of thyroid there is more than 100 per cent increase following heterotransplantation, whereas there is only an approximate increase, varying between 50 and 75 per cent, following homotransplantation.

There is an additional similarity between the character of the local and that of the blood reaction. Loeb [2r] showed that in the case of heterotransplantation a secondary invasion of lymphocytes occurs following the primary action of the polymorphonuclear leukocytes. In my experiments similar changes have been observed in the peripheral blood

reaction. The increase in polymorphonuclear cells is succeeded by a return to normal conditions, reached usually by the tenth day. But soon afterward there occurs an increase in lymphocytes, both absolute and relative, which reaches a maximum between the fourteenth and the sixteenth day, after which the count once more returns to normal.

The wide range in the intensity of the local reaction following syngenesiotransplantation, in which the changes may closely approach those following homotransplantation, on the one hand, and autotransplantation, on the other, is very similar to that seen in the blood cell reaction. Here in some cases of syngenesiotransplantation there occurs an early increase in the number of lymphocytes closely resembling a typical homoreaction, whereas in an instance representing the other extreme the maximum increase was not reached until the nineteenth day. On the average, both local and general syngenesioreactions are of a milder nature than homoreactions; a greater length of time is required until the threshold of effective stimulation is reached, and a longer period is necessary before the height of the reaction is attained.

Since the general reactions so closely parallel the local responses described by Loeb, it seems logical to assume that the mechanism involved is the same in both instances. One may therefore conclude that the transplants give off substances which correspond to the organismal differentials of the graft and that these substances diffuse not only into the area surrounding the transplant but also into the general circulation, where they exert a typical effect on the white blood cells, presumably by the intermediation of the various hemopoietic organs. In accordance with the relationship between the organismal differentials of host and transplant, these substances act as autogenous substances or as syngenesiotoxins, homotoxins and heterotoxins and thus produce their characteristic effect on the host cells.

SUMMARY

Following transplantation of various tissues, changes occurred in the absolute and relative numbers of the various types of leukocytes circulating in the blood which paralleled closely the reactions of leukocytes around the transplants. After homotransplantation this general response was characterized by an absolute and relative increase in lymphocytes; after syngenesiotransplantation the response was similar but, on the average, less intense and reached a maximum after a longer time. After heterotransplantation the primary reaction was of a different kind, being characterized by an early relative and absolute increase in polymorphonuclear leukocytes; but secondarily there occurred here, also, an increase in lymphocytes. By means of these reactions it could be shown that the lens of the eye possessed individuality and species differentials.

In experiments in which successive transplantations were carried out, a more rapid but less intense reaction followed the second transplantation, whether of homogenous or heterogenous tissue, than that which occurred after the primary grafting. The acceleration observed following the second grafting of tissues may have been due to the development of immune substances. If that interpretation should prove correct, it would not imply that the reactions elicited by a single transplant were also due to the formation of antibodies; on the contrary the very rapid onset of this reaction, even after the first transplantation, indicates that it was due to the diffusion of substances from the transplanted tissue into the body fluids. It was possible to prevent the reaction on the part of the circulating leukocytes by injecting trypan blue into the host animal preceding and following the transplantation of tissue. Under these circumstances this dye accumulated around the transplant; to determine whether it acted by obstructing diffusion of substances from the transplant into the host tissues or through blocking the reticuloendothelial system needs further investigation.

In addition a study was made of the effects of temperature and of various chemical substances on the individuality and species differentials which are contained in the transplants, using the reactions of the leukocytes in the circulating blood as indicators. It was shown that the organismal differentials were destroyed by heating at temperatures varying approximately from 54 to 60 C. The exact temperature at which destruction took place was influenced by the density of the grafted tissue. Repeated freezing and thawing did not destroy the individuality differentials but may have caused slight injury in a few cases.

Of the chemical substances tested, only distilled water and 0.9 per cent sodium solution to which thymol had been added failed, as a rule, to destroy the individuality differentials. Isolated instances of nondestruction were observed with a few additional substances. All of the reagents and agencies employed which are known to cause changes in the constitution of proteins were found to be especially active in injuring the organismal differentials.

Whereas transplanted blood clots and plasma clots were able to elicit homoreactions and heteroreactions in accordance with the relationship between transplant and host, as shown by the changes in the distribution of the circulating leukocytes in the blood, injections of heterogenous blood serum failed to induce the heteroreaction. Similarly a number of protein substances of diverse origin failed to elicit the typical heteroreaction; instead, changes in the lymphocytes similar to those seen after homotransplantation occurred. Subjecting these substances to degrees of temperature which destroyed the organismal differentials of tissues did not, however, prevent these reactions. It is therefore probable

that in these cases the reactions were of a less specific nature than those induced by the organismal differentials. In contradistinction to protein substances, carbohydrates and lipoids did not in my experiments exert an effect on the number and distribution of the circulating leukocytes.

CONCLUSIONS

Transplanted pieces of tissues and organs give off substances which bear the organismal differentials and which not only diffuse into the areas of the host directly around the graft but, in addition, reach the general circulation; thus they may be carried to distant places, presumably to the leukocyte-producing organs, and there exert an effect on the number and kind of leukocytes which circulate in the blood. These substances act, therefore, not unlike hormones; it is probable that they are of a protein nature.

APPENDICITIS

I. CHANGES IN THE MUSCULATURE OF THE APPENDIX IN CHRONIC APPENDICITIS

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NEW YORK

This is the first article of a series dealing with the various clinico-anatomic forms of appendicitis. Others will appear later in the course of examination of the accumulated material, which is already considerable.

The study of the musculature of the diseased appendix has been neglected. To state, as do the authors of some comparatively recent reviews on the subject, that the muscular coats of the organ in chronic appendicitis are in certain instances thinned out and in others hypertrophied, and that the muscle cells are concomitantly degenerated, is to take too superficial a view and one that is not based on study devoted specially to the muscle and that is without the advantage of varied microscopic technic and finer histologic interpretation. Moreover, as I shall demonstrate, the thickness of the muscular coats, which varies extremely from one case to another, is in no way a cardinal sign. The musculature of the chronically inflamed appendix offers a multitude of indications which of themselves and independent of changes in the fibrous submucous capsule, lymphoid structures or epithelium permit one to make a definite diagnosis in the majority of instances.

While the aberrations in muscular structure to be reviewed here are characteristic of chronic appendicitis, they are not pathognomonic of it. One may meet with them in acute appendicitis, subacute appendicitis and all the subdivisions of these—even in normal appendixes. Nevertheless, the aberrations in structure are encountered more frequently in chronic appendicitis than in any other condition, their intensity is more striking and their diversity and variability are more marked. Nor should one overlook the fact that aberrant muscle fibers indicate a lesion in the organ to a more marked degree than do the fibrous or epithelial changes. The correlation between the structure of the smooth muscle and the past clinical history is more intimate than is that between the other coats and the symptoms if one excepts the perivascular reactions and the histiocytes.

The changes to be described are capable of furnishing an explanation of the genesis of the disease. Alterations in muscular contractions

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should, of necessity, correspond with the crossed fibers one finds, with the oblique fibers that are rare in normal controls, with the thickenings that constitute true sphincters and with the changes in morphologic arrangement of the coats. Should a spasmodic contraction of a certain intensity and duration arise in one of the areas of tangled muscle fibers or in a pseudosphincter, an entire segment of the organ, controlled by this nodal point, might find itself strangulated; micro-organisms would multiply, toxins be absorbed and cellulitis set up. This is, in short, verification of Dieulafoy's theory of the "closed vessel," not as regards the organ as a whole but rather as regards a segment thereof. That this is true is proved by the observation of certain appendixes in the series investigated which are unchanged in most of their length but show lesions in one segment. Another proof is afforded by the coexistence of muscular and epithelial, fibrous and lymphoid lesions. While muscular changes may exist independently, they are found to be most marked where lesions of the other coats are also most intense.

The lesions of these muscles reveal, at least in part, a very special form of histogenesis of smooth muscle. I have repeatedly confirmed this without, however, being able to find a pathogenic or a physiogenic explanation. A plausible interpretation, which cannot be applied in all cases, in which one demonstrates this histogenesis, is as follows: When one of the muscle coats becomes attenuated or fragmentary or fibrotic, the maintenance of function demands that new muscle fibers with the same orientation as those which have disappeared from the attenuated layer (usually the external longitudinal) should arise in its vicinity and reenforce it or become intercalated among those of the circular coat. It might be that some of these new-formed fibrils offset the abnormal contractions of the coat which has lost its function owing to a variety of lesions. Let me hasten to say that this is merely a hypothesis which cannot at the moment be verified.

The new formation of smooth muscle fibers, wherever situated, does not appear to be accomplished at the expense of preexistent musculature, as will be explained later. It seems rather to be the result of the stimulus of repeated, although abortive, attacks of appendicitis on the histiocytes, whether these are in the lymphoid or in the areolar tissue. This will be discussed at length. It is also important to decide whether these changes in the musculature are a result of such irritation or whether they are congenital. Only a statistical study could give a complete conception of the percentages of aberrant fibers in appendixes of all types and ages. However, aberrant fibers are exceptional in acute appendicitis, rare in the subacute form and frequently seen in chronic appendicitis. Were they already present from birth, one would expect

to find them in a finished, complete state in specimens showing appendicitis, but this is not the case—they are obviously in the process of formation.

The thesis of this work is that the newly formed muscle fibers, by reason of their arrangement and nature, cause rhythmic abnormal contractions of the organ that foster and prolong its pathologic symptoms. That they should be studied from this standpoint is the reason for the present contribution.

MATERIAL AND METHODS

This study is based on the examination of more than 100 appendixes removed in the department of surgery of the New York Hospital, of which about 60 showed chronic appendicitis of the generally recognized types. These have been compared with sections from appendixes showing acute and subacute (in the sense of continued acute) appendicitis and with others from normal appendixes removed incidentally in the course of other operations. Each specimen was received in a fresh condition, immediately placed in a refrigerator and examined about two hours after operation. For this work the apex, the second section taken proximal to it and all but one longitudinal strip were assigned to me, the one distal transverse segment and the single longitudinal strip being used for the departmental routine examination. Often, rolled preparations of the entire organ were prepared, affording sections that represented its entire length. After fixation the segments were sometimes cut into thinner slices and subjected to supplementary fixation.

The following fluids were used for fixation: a formaldehyde-alcohol solution (either 10 parts of solution of formaldehyde U. S. P. to 90 parts of 95 per cent alcohol or Schaffer's formula of 2 parts of from 80 to 95 per cent alcohol to 1 part of solution of formaldehyde U. S. P.); Bouin's solution prepared with trichloroacetic acid; Heidenhain's[1a] Susa fluid (mercury bichloride, saline solution, solution of formaldehyde and trichloroacetic and acetic acids), and Bouin-Susa fluid, which is the last-mentioned fixative saturated with trinitrophenol. Orth's, Zenker's, Helly's and Stieve's fluids were also used. The staining method of choice was my modification of the Masson trichrome light green technic.[1b]

ALTERATIONS OF THE MUSCULATURE

The changes in the morphologic pattern of the muscular coats after repeated attacks of appendicitis are, of necessity, a response to inflammation. For this reason a large variety of pictures may be seen. While it might seem futile to attempt to describe these, a number of definite types of fiber groupings may be recognized and should be put on record for future reference.

Whorls.—The most frequently encountered form of whorl is characterized by fibers running in different directions in the same bundle. Some of these have the orientation one might expect in a given location, while others run obliquely or even spirally (fig. 1). Such whorls may abut on normal fascicles, but they are separated from their surroundings

1. (*a*) Romeis, B.: Taschenbuch der mikroskopischen Technik, Munich, R. Oldenbourg, 1932. (*b*) Goldner, J.: Am. J. Path. **14**:237, 1938.

by reticular or collagenous fibers. Often they are isolated by enveloping muscle fibers that course in a direction opposite to that proper to the fascicle. Originating in the interior of the whorl, they leave it in the form of wicklike bundles that proceed more or less to encapsulate it. They may join with similar bundles from other whorls to enmesh groups of whorls or to form a median, rather incomplete layer between two muscle coats (figs. 1 and 2). Their course after leaving the whorl may be devious.

These whorls may be grouped into masses (fig. 3), which may replace an entire region in one of the muscle coats, most often the

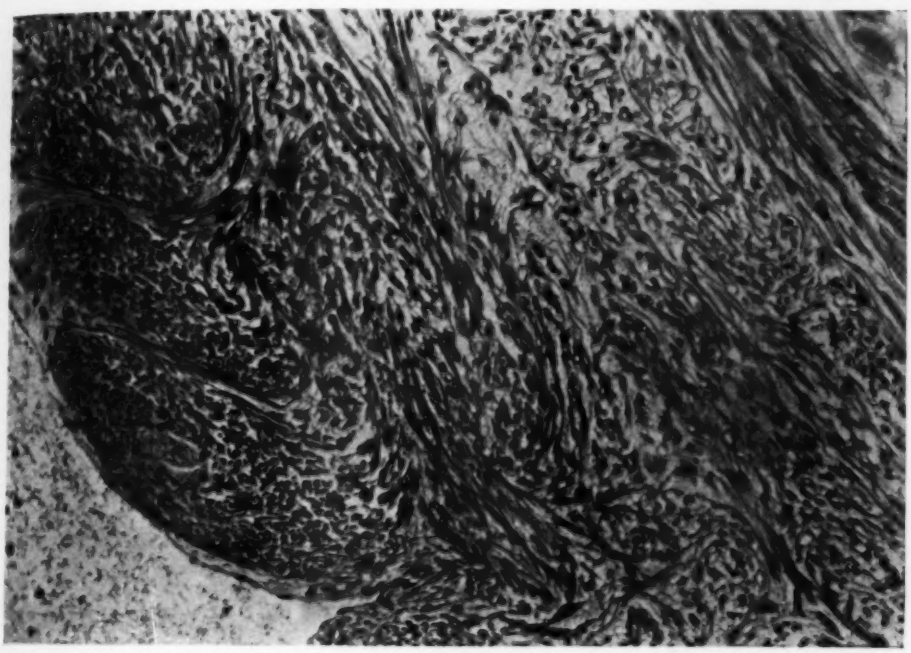

Fig. 1.—Chronic sclerotic appendicitis ab initio; transverse section. Note whorls, the coexistence of fibers running in different directions and enveloping fibers.

external, or may become intercalated between the two coats, forming a supplementary, or middle, coat. By their presence they recast the appearance of entire regions, whatever may be the histiologic makeup, and distort the normal topography.

Aberrant Fibers.—(*a*) Mixed Fascicles: These are usually situated in the external coat. Their dimensions are slender, and they are characterized by the coexistence within them of longitudinal and transverse fibers. Oblique and spiral forms are wanting. They do not appear to

produce penetrating fibers that leave the bundle to connect it with a layer running in the opposite direction. They rarely show enveloping fibers, which like the penetrating fibers are characteristic of the whorls. The mixed fascicle may be separated from similar bundles by connective tissue and tends to lie out of alinement, forced into a blind corner by neighboring nerve plexuses, vessels and the like. Everything indicates that it has an autonomous function.

(*b*) Mixed Fascicles with Interlacing Fibers: They are rarely seen. They occur only in the circular coat and may be so extensive as to occupy two low power fields. Their chief feature is their intricate weave.

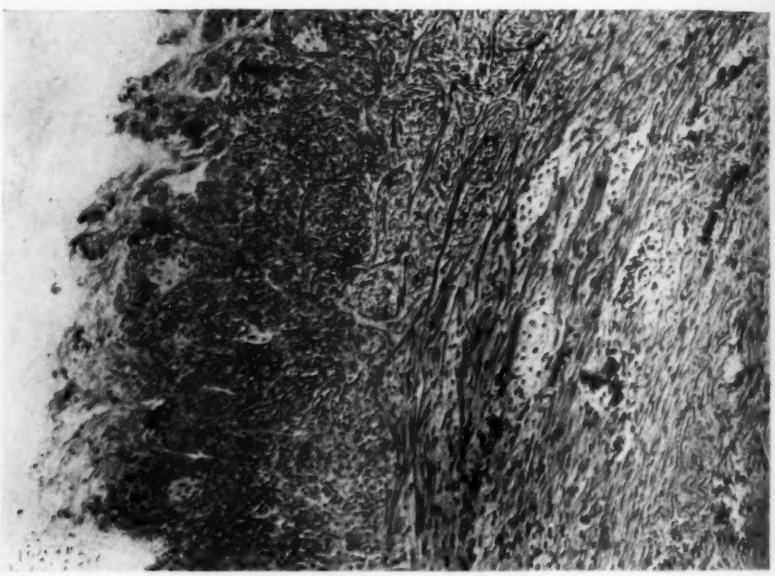

Fig. 2.—Chronic hyperplastic appendicitis ab initio; transverse section. There is a supernumerary middle layer between the external and the internal coat, formed by transversely running fascicles and surrounded more or less completely by circular or spiral fibers. The circular fibers trail off into narrow and isolated fascicles.

There are areas where fibers of one direction predominate over those running in another, in spite of their interlacing topography.

(*c*) Mixed Fascicles in Isolated Groups: These are characterized by their form, isolation and deeper staining affinities. They are usually situated in the circular coat and are most readily found in longitudinal sections. They are ovoid, and most of the fibers composing them run in the direction of the coat in which they lie. They tend to be coarser, to stain more deeply and to show a larger number of nuclei (fig. 4).

(*d*) Crosses: I have mentioned the "penetrating fibers" that leave the whorls to encase them or to mingle with similar fibers from other whorls; frequently they cross these, forming maltese crosses with a narrow point of intersection and flaring arms. The fibers may cross at an angle at the point of intersection or may present merely two loops that approach each other closely, with or without exchange of fibrils, to form a spencerian letter X (fig. 3).

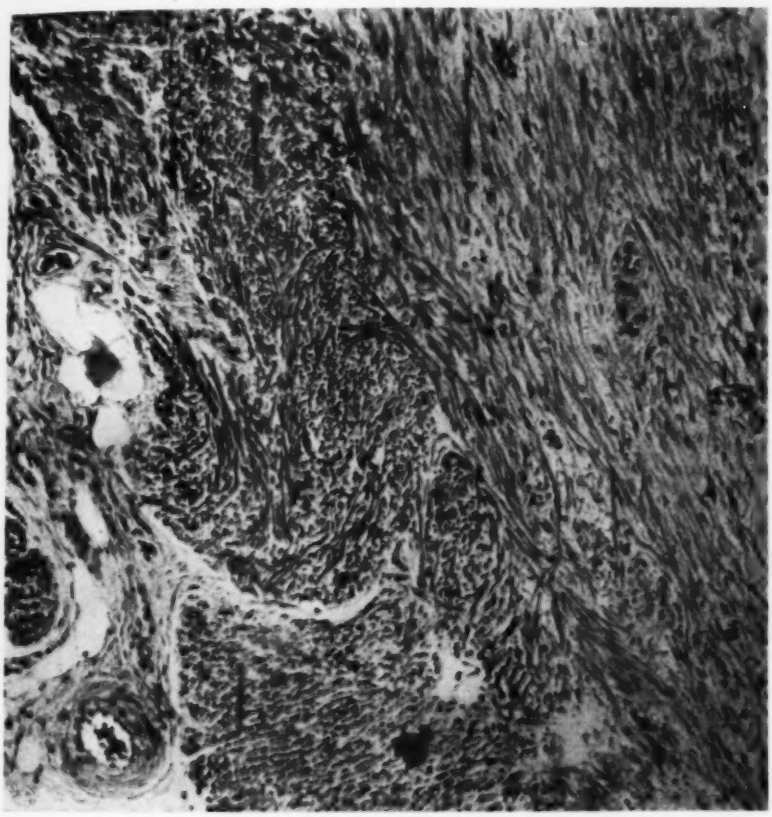

Fig. 3.—Chronic ulcerative appendicitis; transverse section. A group of whorls may be seen. At the left, near the fat cells, are fibers penetrating into the fascicles of transverse fibers (external coat). Near the middle are three packets of transverse fibers enveloped by longitudinal fibers. Near the right are intersecting longitundinal fibers.

(*e*) Matted Fibers or "Herring Bones": These show fibers that intersect one another at acute angles and are more or less curved or even curled. The spaces left in this tangle are occupied by ganglion cells, nerves, capillaries and other structures; more important, there may be bundles of muscle fibers in them which run in curves.

(*f*) Bridge Fibers: These may be composed of a single fiber or of several, and broader or coarser ones may serve to join two muscle layers or portions thereof. They exist in large variety and have been noted arising from the subserosa, possibly reenforcing gaps in the external coat, but running counter to it. They may pass to the circular coat and where it has been cleft by vessels or other structures may be seen running across the cleftlike stays. Finally, they may unite the circular coat with the muscularis mucosae. They are sometimes seen to take a wavy, arabesque-like course and to occur multiply rather than singly; they may then be called arabesque fibers.

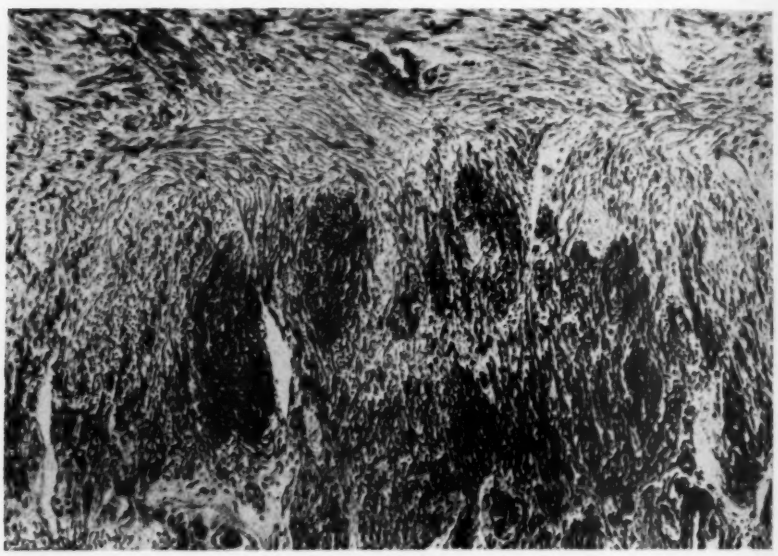

Fig. 4.—Chronic appendicitis; longitudinal section. There are mixed fascicles in isolated groups. Besides these deeply staining ovoid formations, note interlacing fibers.

(*g*) Loops: Fibers may arise in the circular coat, follow its course for a while after leaving it and then run out of the section. Serial sections, however, prove that after leaving the coat and describing a loop, they return to the circular coat. Another variety of loop is furnished by recurrent fibers that are a form of enveloping fibers, which leave their fascicle, the bundle increasing in thickness by reason of the addition of other fibers, and run obliquely or in curves to the internal coat, where they may or may not exchange a few fibrils (fig. 5). They then course back to the external coat and in so doing embrace whorls with multidirectional fibers, forming a true supernumerary layer of aberrant fibers.

(*h*) Penniform Fibers: These are another form of enveloping or penetrating fibers that are found in multiples rather than isolated. From each whorl in a given region, or from its capsule, fibers run obliquely toward a more or less isolated fascicle or toward the circular coat. This forms the shaft of the feather; the oblique fibers that join it, the barbs.

(*i*) Digitiform Expansions: These arise in the most central portion of the circular coat and are distributed through the submucous connective tissue, their destination usually being the muscularis mucosae. They are common in chronic appendicitis undergoing fibrous obliteration. They

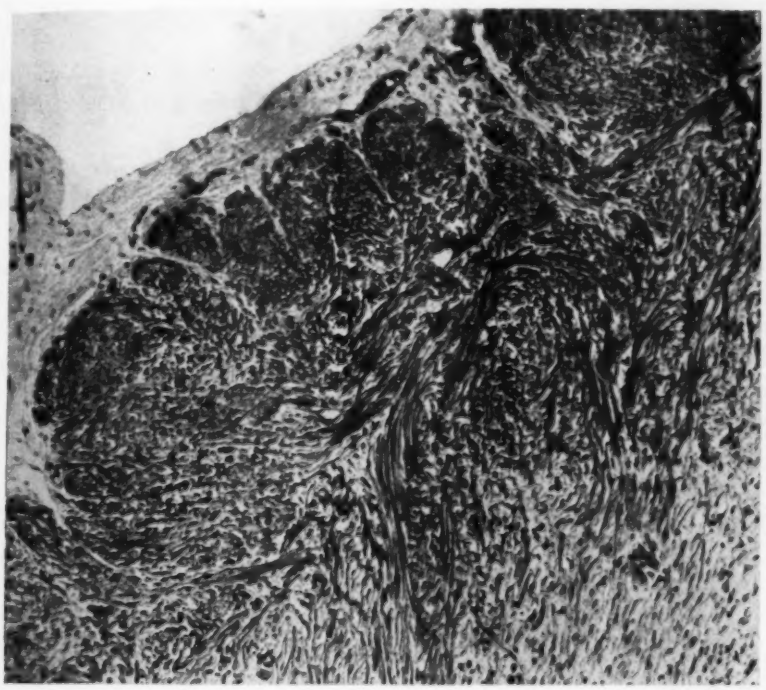

Fig. 5.—Chronic fibrous appendicitis; transverse section. Several groups of transverse fibers may be seen in the internal layer. Each packet is enveloped by longitudinal fibers, which reunite to outline these groups and then lose themselves after turning aside to the internal layer. Recurrent fibers are seen generally throughout the field.

run at first parallel to the circular coat, diverging obliquely and possibly decussating with other fibers; usually they form patterns like hands with outstretched fingers. Whorls may be formed in these digitations. The digitiform fibers may form blunt processes extending toward the muscularis mucosae without reaching it, or they may return to the fascicle whence they arose in the form of recurrent fibers. If they

encounter more or less rigid anatomic structures in their path, they may split to surround these, reuniting when the obstacle is passed.

Fate of Aberrant Fibers.—Of these the whorls, whorled fibers and supernumerary layers give one an impression of permanency; the other forms seem to be in a state of evolution and rearrangement. There appear to be processes of evolution going on in one part of them, while in another segment histolysis is taking place. Points of sarcolysis are, however, rare. The lytic processes are the work of histiocytes.[2]

CHANGES IN THE MORPHOLOGIC APPEARANCE OF THE MUSCULAR COATS PROPER

The muscular coats as such may show variations within themselves: whorls and aberrant fibers, changes in thickness and compensatory alterations.

Changes in Thickness.—The thickness of the muscular coats is extremely variable; the normal ratio between the external and the internal coat, maintained in acute appendicitis, is lost in the chronic condition. Most striking is the variability of thickness over long stretches, which may involve one or both coats. This is best noted in rolled sections of the entire organ, the longitudinal section giving more scope. Where one coat is thinned the other does not necessarily become thickened, as newly formed fibers that are strewn between the coats or in the submucous layer of connective tissue take care of compensation. In transverse sections there is sometimes noted a thinning in one semicircle of the organ which is counterbalanced, as it were, by a corresponding thickening in the same coat but in the other semicircle and on the opposite side of the organ. Numerical ratios vary tremendously; the inner coat may measure from 6 to 1, while the outer varies from 3 to 2.

Suppression of Coats; Occurrence of Supernumerary Coats.—The external coat may be fragmentarily wanting (fig. 6); it may be supplemented by arched or oblique aberrant fibers (fig. 7) or by connective tissue from the subserosa. When it is lacking, the internal coat is found to contain whorls, aberrant fibers or fibers that run in the same direction as those of the missing external coat. The transitions between the normal and the suppressed or aberrant region of a coat may occur abruptly or gradually. In the latter case, one sees successively fibers that run in all directions and form whorls; next, simpler packets of external coat and oblique fibers which trail off into subserous projections. At such points the external and internal coats become solidly fused, their fibers interlacing obliquely or at right angles.

2. Goldner, J.: Compt. rend. Soc. de biol. **144**:1131, 1933.

In other cases, the external coat instead of disappearing, appears to be formed of sparsely scattered fascicles of fibers. A majority of these have the appearance of fibers of the internal coat, running obliquely.

The supernumerary coat may involve either coat. The circular coat may be reenforced by increased connective tissue or by islets of muscle scattered through the submucous fibrous capsule, sometimes uniting the internal coat to the muscularis mucosae. The reenforcing fibers of the external coat may appear as islets with fibers running variously, astride the coat, scattered between it and the serosa or even invading

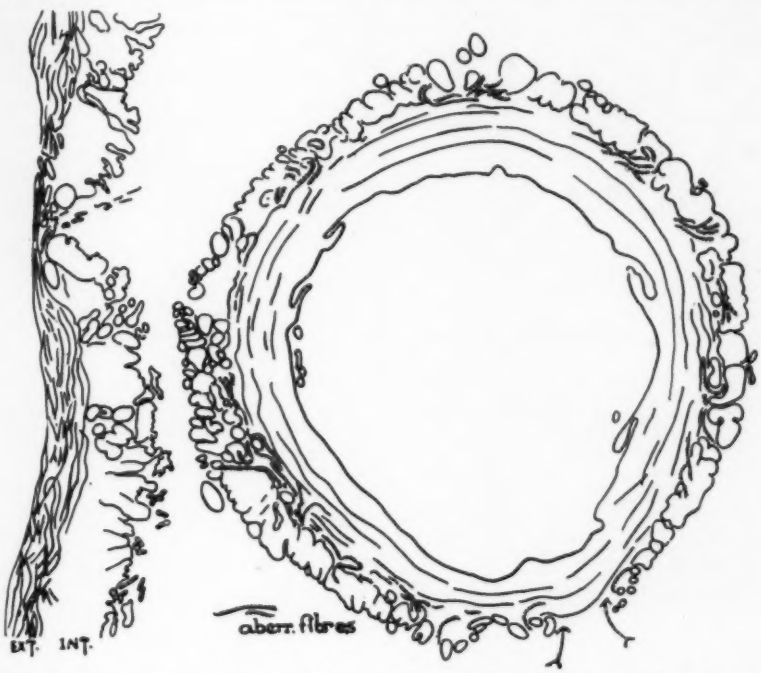

Fig. 6.—Diagram at left (chronic ulcerative appendicitis; longitudinal section): Note the variations in the thickness of the two coats. At one point the very thin external coat is reenforced by packets and fascicles from the internal coat. At this point digitations of longitudinal fibers run into the connective tissue. Notice also the intersections of the fibers of the external coat.

Diagram at right (chronic lymphoid appendicitis; transverse section): Note the variations in the thickness of the two coats. Between the two arrows one may note the disappearance of the external coat, which occurs gradually, stout packets being replaced by smaller and smaller fascicles. Note the geographic outline of the internal margin of the inner coat, the course of the aberrant fibers and the occasional transverse fibers that lie in the sublymphoid connective tissue.

the serosa. They may appear as isolated nodules which have nothing to do with the muscle itself, being completely separated from it. There are no bridge fibers here.

Another variation is intercalated fibers. Instead of showing two distinct layers, certain appendixes with chronic inflammation show first, an outer longitudinal or slightly oblique coat, a transverse coat, where the muscle bundles appear to be forced into the outer coat. This is succeeded by another longitudinal or slightly oblique coat and finally by the circular coat.

HISTOGENESIS OF ABERRANT MUSCLE FIBERS

I have never observed mitoses or amitotic division in the fibers of the two muscular coats or in the vascular cells, and classic myoblasts have been totally wanting. The only cell which could, then, produce an aberrant muscle fiber is the histiocyte, more particularly the adventi-

Fig. 7.—Chronic fibrous appendicitis; transverse section. The external coat is replaced by oblique fibers forming loops. The oblique fibers mingle with the circular fibers of the internal coat.

tial histiocyte of Marchand. In this respect the data presented here differ from those of Stieve,[3] who believed that lymphocytes emigrated from the vessels of the pregnant uterus and became histiocytes which multiplied and produced muscle fibers, or that fibroblasts might accumulate early in pregnancy to produce muscle fibers later. He believed these were also of histiocytic origin. It is not evident in my sections that the lymphocytes (which are abundant) are ever transformed into muscle cells.

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3. Stieve, H.: Ztschr. f. mikr.-anat. Forsch. **17**:371, 1929; Zentralbl. f. Gynäk. **53**:2706, 1929.

In drawing general conclusions as to this histogenesis one may point out:

1. The arrangement of newly formed muscle cells imitates that of newly formed capillaries (fig. 8). The capillary appears to form a sort of core for the production of muscle fibers; it may disappear, for many such vessels are merely temporary in function, and their development may remain uncompleted (e. g., there may be absence of an endothelial

Fig. 8.—Newly formed muscle fibers the arrangement of which suggests that of building capillaries.

lining). Their temporary morphogenesis seems to afford a form of preparation for the histogenesis of muscle fibers.

2. New muscle bundles, like new vessels, employ the interstices of the connective tissue as formative molds.

3. Muscle fibers may pass from one capillary to another, sometimes constricting them and causing them to disintegrate and cease to function (fig. 9).

4. The new formation of muscles stimulates proliferation of reticulum, reticular fibers determining the direction of the muscle fibers and separating them from each other (fig. 10 *A*, *B*, *C* and *D*).

5. The earliest muscle fibers appear as sleeves around the capillaries; by the time that they have been formed there are no adventitial cells left, so that no more muscle can be produced. These muscle cells do not divide; they merely grow in size. Fibers connecting them with other groups are produced by histiocytes in the intervening reticular layer (fig. 10 *E*).

It has not been possible to follow every stage in the transformation indicated in the foregoing paragraphs, nevertheless one may deduce that

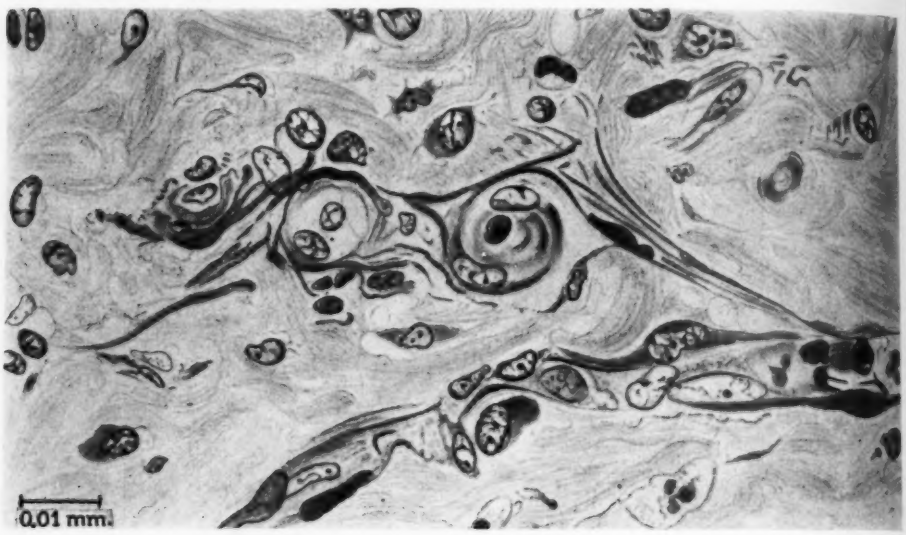

Fig. 9.—Capillaries undergoing evolution. At the right, a capillary has disappeared (neither endothelium nor perithelium being visible); sparse muscle fibers envelop it partially. In the capillary that runs longitudinally (below) there are endothelial and perithelial reactions. One of the histiocytes has produced fibrils. The other is elongated into a spindle; its cytoplasm is dense and darkly staining. The three capillaries that run transversely are compressed by the smooth muscle fibers that course among them. The adventitial cells show fibrils. The neighboring histiocytes, increased in number, are of the reacting type, some of them showing fibrils. Supporting fibers connect transverse with longitudinal capillaries.

the disintegration of capillaries sets free the adventitial cells, which become more like Maximow's polyblasts as the process goes on, and that these cells may begin producing myofibrils even before they have assumed a fusiform character (fig. 9). These phases can be seen in my sections.

Fig. 10.—Capillaries at the time of formation of smooth muscle fibers: In *A* note the narrowed lumen, the metamorphosing endothelial cell and the altered perithelial cells containing fibrils. Reticulum separates the cells from one another. In *B* note the narrowed lumen, the fragments of erythrocytes, the desquamating endothelial cells, the outlining of the lumen by reticulum and the histiocytes with a fibrillary structure. In two of the latter the fibers run transversely, while those in neighboring cells run in the opposite direction. *C* shows an obliterated lumen bordered by reticulum. The muscular fibers run transversely. The fascicle is enveloped by smooth muscle fibers that emanate from the perithelial cells. The evolution of one of the perithelial cells is retarded (plump nucleus, rare fibrils, a reacting type). *D* shows disappearance of the endothelium, with effacement of the lumen. The reticulum that surrounds it joins that which separates the muscle fibers from one another. Smooth muscle fibers run in two directions. Note the evolutionary phase of the adventitial cells. Nearby is a plasma cell. *E* shows capillaries running longitudinally. Note the metamorphosis of the endothelium (desquamation; monocytic and polyblastic appearance). The endothelial tube is ensheathed by muscle cells through a transformation of the perithelial tube. Some adventitial cells of the reacting type are seen below. A second file of muscle fibers is apposed (at right); their origin is histiocytic. Note the reacting phase of the histiocytes of the reticular network. In this figure the scales shown are all drawn to 10 microns.

If one follows the direction of the myofibrils contained in these adventitial cells in the course of transformation, one finds them running longitudinally in some, transversely in others, in their immediate vicinity (fig. 10 *B*). Thus, the muscular sheaths of some capillaries are composed of fibrils running in two opposed directions. Part of these fibrils surround the vessel as long as it is functional; the more peripherally situated cells, however, leave the vessel and push out along its longitudinal axis (fig. 10 *E*). The new muscle fibers may remain more or less as they are, or they may increase in number and form bundles, the increase being the result of a transformation of histiocytes, which also form reticulum and direct the course of the muscle fibers, rather than of a proliferation from adventitial cells.

Fibers arising from capillaries that run at angles to the normal muscular coats deviate from the vascular axis at once and retain this deviation. Instead of reenforcing one of the coats, they become aberrant fibers of the bridge or loop type. Their direction is first dependent on that of the capillary; later it is determined by the tissue spaces, where the capaciousness allows the formation of arabesques and where the contractions of the altered muscular coats may force the aberrant fibers into new courses.

Muscular fibers are not formed alone at the expense of the capillaries that course in the muscular coats; they are also derived from those that lie in the connective tissue separating these coats from each other or from the submucous fibrous capsule. In the latter instance, the fibers that arise constitute the aberrant musculature that forms the median intercalated coat, digitations of which join the muscularis mucosae or whorls and groups of fibers that represent the displaced remains of attenuated anatomic coats that are in regression.

CONCLUSIONS

In chronically inflamed appendixes there are morphologic deviations of the two muscular layers, with attentuation or thickening, disappearance and the production of supplementary fibers.

Aberrant fibers appear ectopically, forming median coats, surrounding lymph follicles or lying in the fibrous capsule of the submucosa.

The structural changes described here are frequently seen in chronic appendicitis, fairly frequently in the subacute inflammation that signalizes early chronic appendicitis and very rarely in acute appendicitis and in normal conditions of the appendix. Their presence denotes past attacks that have stimulated the production of capillaries and histiocytes. They exist even when other cardinal signs (notably those proper to the lymphoid apparatus and epithelium) have subsided.

Functional changes in the form of abnormal contraction should correspond to these structural abnormalities of the musculature, which would in part explain the pathogenic mechanism of this disease. Abnormal contraction may be as important etiologically as changes in the rigid connective tissue framework, which also shows lesions of past attacks.

Aberrant fibers, as well as those that cause the thickening of one or the other coat, have a special histogenesis; their parent cell is the histiocyte, more particularly the adventitial cell of Marchand.

Preformed or newly formed capillaries disappear.

Although the majority of aberrant muscular structures appear to be permanent, others may fall prey to sarcolysis or to rearrangement. The lytic forces involved are supplied by histiocytes.

Case Reports

RETICULOENDOTHELIOMATOSIS

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NEW YORK

In the Laboratories of Pathology at Bellevue Hospital we have had occasion to study a neoplastic disease involving not only those organs entering into the formation of the reticuloendothelial system—the spleen, lymph nodes, bone marrow and liver—but also structures not customarily regarded as members of that system, among them, the pericardium and pleura, both adrenals, the pancreas, the small intestine, the gallbladder, both kidneys, the uterus, fallopian tubes and ovaries, and the thyroid. The individual tumors are characterized histologically by an overgrowth of minute channels lined by endothelial cells provided with delicate spine-like processes which are impregnable by silver. The spleen was massive, and the lymph nodes were enlarged to a remarkable degree. The bone marrow was replaced over a wide expanse by tumor growth, and the patient presented the picture of a variety of myelophthisic anemia. We were unable to find a description of any comparable condition in the literature of medicine, and the disease is believed to be unique. It is best designated, we think, as reticuloendotheliomatosis.

REPORT OF A CASE

A white woman aged 42 was admitted to Bellevue Hospital to the medical service of Dr. Charles E. Nammack, Nov. 6, 1933, and died March 5, 1934. She complained that for eight months she had suffered from constant generalized pains referable to the bony system. She described the pains as "drilling." She stated that two months prior to examination she had noticed masses in both armpits and a tender mass in the upper part of the abdomen on the left side. She said that she had lost 40 pounds (18 Kg.) in the past year, and she complained of shortness of breath.

The patient was obese. The skin was flabby and presented a diffuse lemon yellow tint. Petechiae and small ecchymotic extravasations were visible in the skin covering both arms and legs. The cervical, axillary and inguinal lymph nodes were enlarged. The spleen was palpable at the crest of the left ilium and was tender. The edge of the liver was felt 6 cm. below the level of the costal slope in the right midmamillary line. The organ was tender. Palpation elicited tenderness throughout the bony system. Both legs were markedly edematous. The patient was dyspneic. The temperature ranged between 99 and 100 F.

From the Laboratories of Pathology, Bellevue Hospital.

Blood counts made over a period of several months averaged 1,500,000 red cells. The hemoglobin content ranged from 25 to 30 per cent. The white cells numbered on the average 20,800 per cubic millimeter, with a high percentage (82) of polymorphonuclear neutrophils. There were many normoblasts, megalo-

Fig. 1.—High power photomicrograph of a lymph node, showing minute channels lined by reticulum cells with spinelike processes; silver impregnation.

blasts and macrocytes, a few reticulocytes, some anisocytosis and an occasional myelocyte. The color index varied from 1 to 1.13. The platelet count averaged

14,430; the coagulation time, one and a half minutes; the bleeding time, five and a half minutes. The icterus index was 5. The urine on repeated examination was normal.

Roentgen examination showed rarefaction and sclerosis in the rami of the pubic bones, in the ilia and in the upper third of the femurs, rarefaction of the skull and sclerosis of the lumbar region of the spinal column. The changes were interpreted as those of a metastatic malignant tumor, probably carcinoma.

Fig. 2.—Low power photomicrograph of spleen, showing interlacing reticulum fibers dividing the pulp into small islands; silver impregnation.

Microscopic examination of an excised inguinal lymph node showed changes which were interpreted as those of a metastatic adenocarcinoma.

Necropsy.—The incision was limited from the xiphoid cartilage to the pubic symphysis.

The body was that of an obese white woman 155 cm. in height and weighing 102 Kg. There was marked edema of the lower extremities, the left hand and

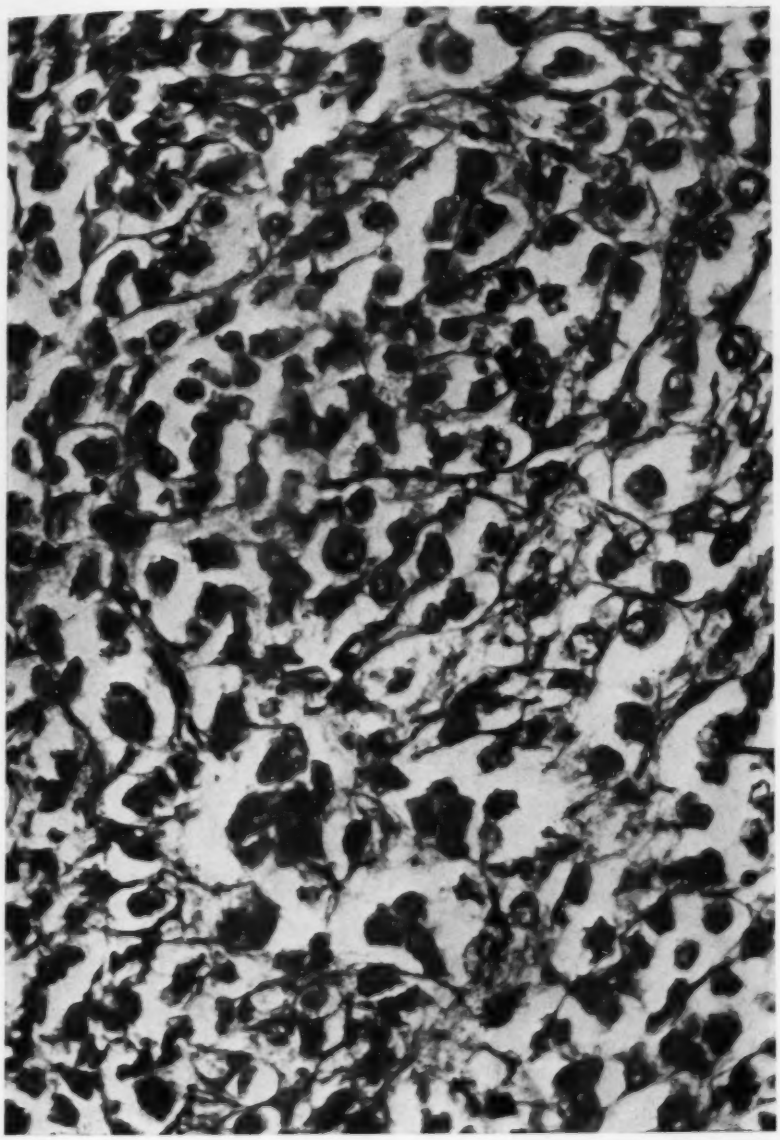

Fig. 3.—High power photomicrograph of spleen, showing minute channels lined by reticulum cells with spinelike processes; silver impregnation. Compare with figure 1.

the region of the right breast. The fat in the anterior abdominal wall was abundant; the muscle tissues were atrophic. The peritoneum was smooth, and the sac contained about 2,000 cc. of thin straw-colored fluid. Embedded in the pericardium, which for the greater part was smooth and glistening, were numbers of grayish dome-shaped nodules, sharply demarcated, sessile and from 1 to 3 mm. in diameter. The pleura of both lungs revealed extensive smooth grayish opaque

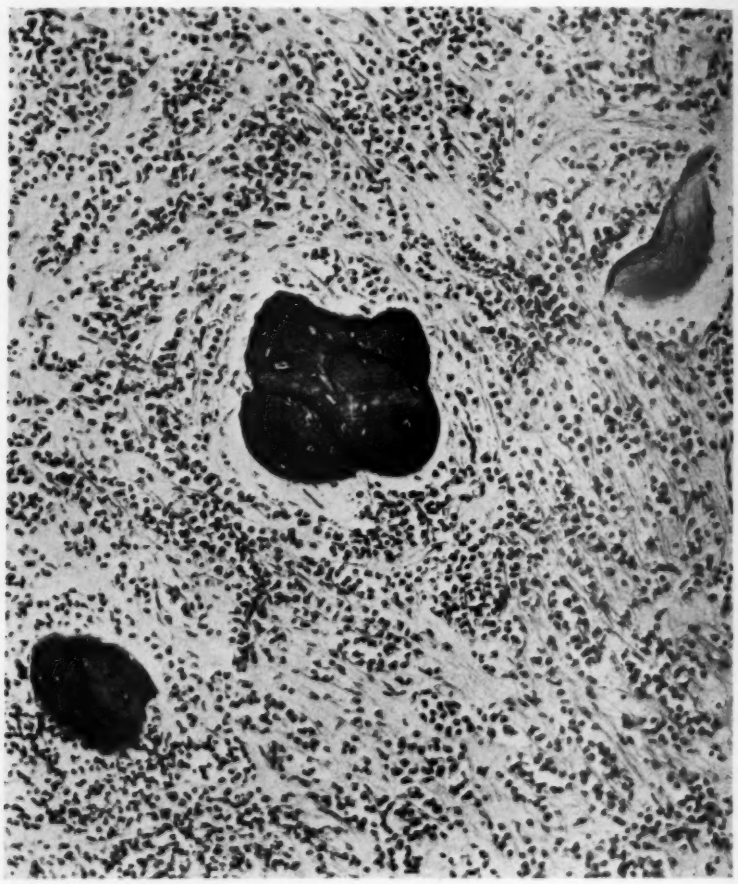

Fig. 4.—Low power photomicrograph of bone marrow, showing the formation of delicate canals and the abundance of intercanalicular substance; hematoxylin and eosin.

areas of thickening and numerous nodules of the same size and character as those described in the pericardium, together with delicate grayish linear projections into the substance of the membrane. The spleen weighed 1,150 Gm. The organ was red and tough, the capsule smooth. On section the substance was riddled with ill defined opaque grayish foci, measuring from 3 to 6 mm. in diameter. The liver weighed 3,075 Gm. Scattered through the capsule were moderate numbers of grayish, dome-shaped nodules from 2 to 15 mm. in diameter. Strewn through the substance were nodules of the same character as those described

in the capsule. The walls of the gallbladder contained a few nodules of similar appearance, averaging about 6 mm. in diameter. The pancreas also showed moderate numbers of nodules of much the same size and appearance. The adrenals were greatly increased in size and riddled with poorly defined grayish nodules varying in size from 1 to 4 cm. Scattered through both kidneys were a few nodules, measuring about 2 mm. in diameter and differing in no essential from

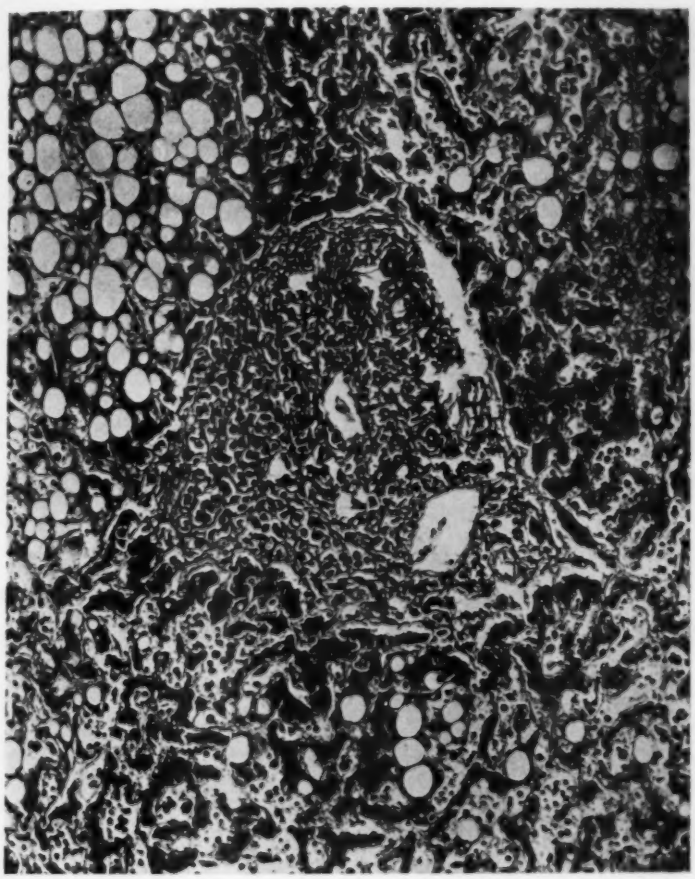

Fig. 5.—Low power photomicrograph of liver, showing atrophic and fatty parenchymal cells and interlobular collection of minute channels lined by reticulum cells; silver impregnation.

those encountered elsewhere. Embedded in each ovary was a solitary nodule, 1 cm. in diameter, of the same description as those referred to. The walls of the fallopian tubes were thickly strewn with nodules, all about 2 mm. in diameter. Two subserous sessile nodules, measuring, respectively, 3 and 8 mm. in diameter, were present in the fundus of the uterus, and several nodules, about 4 mm. in diameter, were buried in the endometrium. The cervical, peribronchial, anterior mediastinal, mesenteric and pelvic lymph nodes were enlarged, varying in diameter from 1 to 3 cm. The retroperitoneal nodes were extensively involved, and those

in the celiac region formed a mass 10 cm. in diameter. The cut surfaces of the lymph nodes presented a grayish opaque homogeneous appearance with faint undulations. The marrow of the pubic bones, lumbar vertebrae and ribs was practically completely replaced by firm grayish opaque tissue. In the upper part

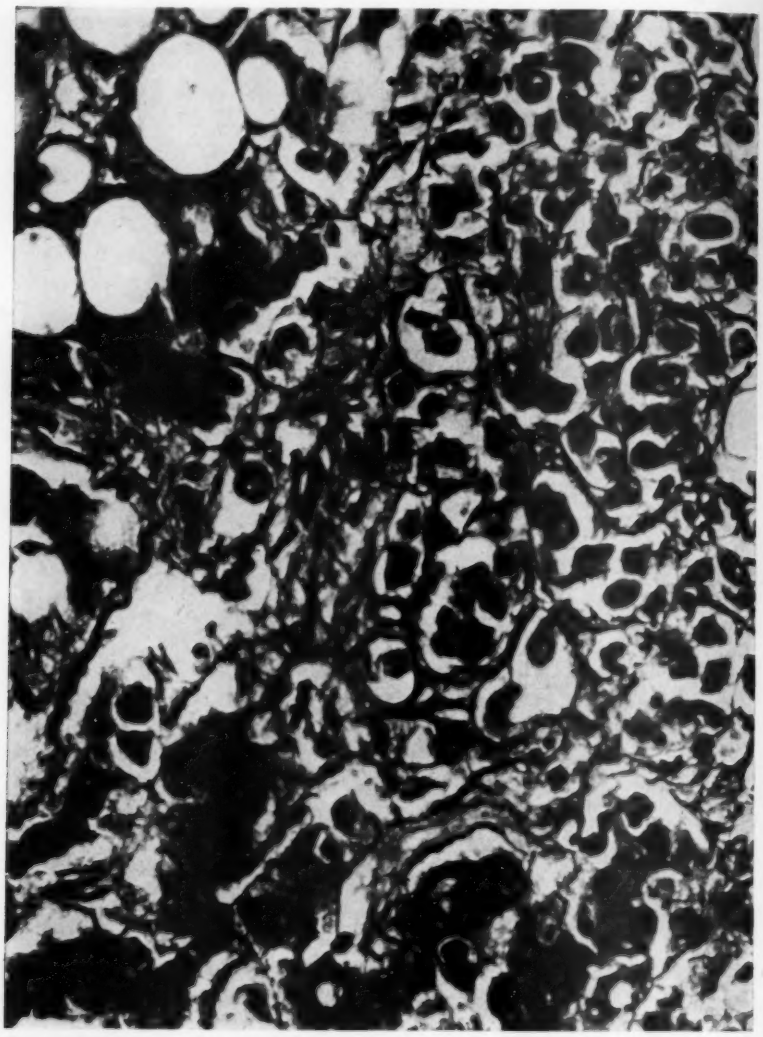

Fig. 6.—High power view of a part of the area shown in figure 5. Note atrophic and fatty parenchymal cells and minute channels lined by reticulum cells with spinelike processes; silver impregnation. A few reticulum fibers are present.

of the right lobe of the thyroid were two firm grayish nodules, each measuring about 1 cm. in diameter. Permission for examination of the head was not obtained. Other than the changes described, necropsy revealed nothing worthy of record in the present connection.

Histologic Observations.—Lymph Nodes: These were partially or completely replaced by an overgrowth of capillary channels, limited externally by threadlike membranes, lining which were continuous or broken layers of irregularly shaped cells containing scanty, slightly acidophilic cytoplasm with delicate spinelike processes. These cytoplasmic processes were not infrequently to be seen in sec-

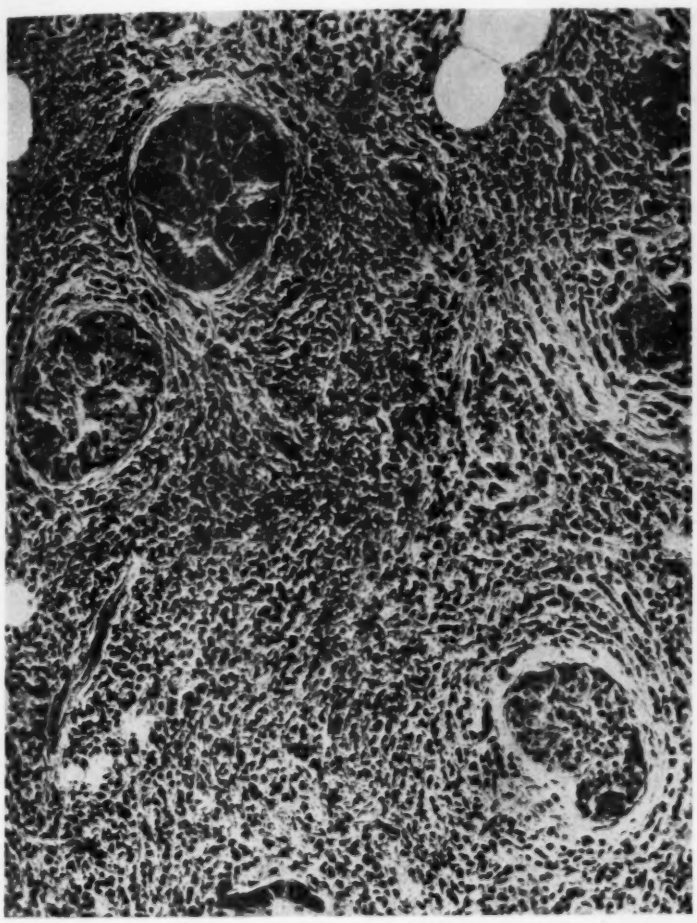

Fig. 7.—Low power photomicrograph of pancreas, showing three islands of Langerhans surrounded by reticulum cells arranged concentrically or in linear formation; hematoxylin and eosin.

tions stained with hematoxylin and eosin but were accentuated when impregnated with silver (fig. 1). In some instances the capillary growths extended into the capsule and perinodal fat tissues. In many instances the capillary channels were partially blocked or even packed to the point of distention by detached cells identical in morphologic character and staining characteristics with those just described.

Spleen: The architecture was practically completely obscured by an overgrowth of pinkish-staining, poorly nucleated tissue, which divided the substance of the organ into islands of different shapes and sizes and of variable cell content. In sections impregnated with silver (fig. 2) this supporting tissue was seen to consist of a dense network of reticulum fibers. In places it surrounded islands of pulp and sent individual reticulum fibers into the pulp. In other places were islands made up of capillary vessels lined by argentophilic endothelium with spinelike processes. These cells were scarcely distinguishable from the tumor cells in the lymph nodes (fig. 3). In some places the hyalinized walls of an arteriole were visible, and the insular structure as a whole was thus recognizable as an altered lymph follicle.

Bone Marrow: Many of the interstices in the bone marrow were filled with capillary channels lined by endothelial cells with argentophilic cytoplasmic processes. These cells were of the same appearance as the tumor cells in the lymph nodes and spleen (fig. 4). In silver preparations the intercanalicular tissue was shown to consist of interlacing reticulum fibers. In other interstices were closely crowded marrow cells whose identities could not always be distinguished in paraffin sections, although eosinophilic myelocytes were abundant and megakaryocytes were occasionally discernible.

Liver: The lobular formation was lost as was the columnar arrangement of the parenchymal cells. The latter were distorted and atrophic to an extreme degree, and many of them were coarsely vacuolated (fat). The sinusoids appeared to be greatly widened. The Kupffer cells were unchanged. In the perilobular connective tissues were numerous collections of capillary vessels which in sections stained with hematoxylin and eosin and in sections impregnated with silver were apparently identical with those in the lymph nodes, spleen and bone marrow (fig. 5). The foci in question were well circumscribed and were often irregular or spider-like in contour, the capillary vessels extending for short distances between immediately adjacent liver cells (fig. 6). Such foci varied in size from islands of microscopic dimensions to those which replaced one or many lobules.

Pericardium, Adrenals, Kidneys, Small Intestine, Pancreas and Uterus, Including Myometrium and Endometrium: The histologic picture in all these tissues was essentially identical with that in the lymph nodes, spleen, bone marrow and liver, but all of the former organs revealed in addition to the overgrowth of capillary vessels lined by endothelial reticulum cells the same sort of reticulum cells distributed in solid rows running parallel with one another or arranged concentrically (fig. 7).

COMMENT

It is our impression that the neoplastic disease described in this paper under the designation of reticuloendotheliomatosis is unique and that it is a clinical and pathologic entity. It is characterized (1) by multiple small nodules in various organs, including not only those of the reticuloendothelial apparatus but also the pericardium and pleura, both adrenals, the pancreas, the small intestine, the gallbladder, both kidneys, the uterus, fallopian tubes and ovaries, and the thyroid, (2) by growth units consisting of minute channels lined by argentophilic endothelial cells with spinelike processes, and (3) by a variety of myelophthisic anemia due to widespread neoplastic replacement of the bone marrow.

HEMANGIOENDOTHELIAL SARCOMA OF THE THYROID WITH EXTENSION INTO THE TRACHEA AND WITH MASSIVE HEMOPTYSIS

MORRIS A. SIMON, M.D.,* MONTREAL, CANADA

Hemangioendothelial sarcoma is an uncommon tumor of the thyroid. At the present time the literature reveals reports of 21 cases, only 1 of which was published in America. The condition is not so infrequent as the figure stated would indicate, for Wegelin [1] noted the appearance of 19 cases of this type of tumor in his material at Bern, Switzerland, during the period from 1907 to 1922. A further review of the material at Bern (from 1922 to 1938) by me reveals 22 additional instances. The cases reported are distributed as follows: Hedinger,[2] 6 cases; Limacher,[3] Frattin [4] and Clivio,[5] 2 cases each, and Usui,[6] Winnen,[7] Matti,[8] Blum,[9] Distefano,[10] Wülfing,[11] Kassel,[12] Ponhold [13] and Rice,[14] 1 case each.

As the name indicates, the tumor is believed to arise from proliferating endothelium of blood vessels, and in its growth it simulates the formation of small vessels and capillaries. The morphologic appearance of the cells comprising the tumor also points to endothelial derivation.

From the Pathologisches Institut, Berne, Switzerland, Prof. Dr. C. Wegelin, director.

*E. E. Wolf Fellow in Pathology, Institute of Pathology, Western Reserve University, Cleveland.

1. Wegelin, C., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 8, pp. 297-302.

2. Hedinger, E.: Zur Lehre der Struma sarcomatosa. I, Frankfurt. Ztschr. f. Path. **3**:487, 1909.

3. Limacher, F.: Ueber Blutgefässendothelien der Struma, mit einem Anhang über Knochenmetastasen der Struma maligna, Dissert., Bern, Berlin, 1898.

4. Frattin, G.: Arch. per le sc. med. **25**:138, 1907.

5. Clivio, R.: Pathologica **14**:759, 1922.

6. Usui, T.: Berl. klin. Wchnschr. **44**:1975, 1911.

7. Winnen, P.: Frankfurt. Ztschr. f. Path. **23**:405, 1920.

8. Matti, H.: Schweiz. med. Wchnschr. **65**:59, 1935.

9. Blum, W.: Schweiz. med. Wchnschr. **66**:781, 1936.

10. Distefano, G.: Zentralbl. f. allg. Path. u. path. Anat. **67**:70, 1937.

11. Wülfing, H. W.: Ztschr. f. Krebsforsch. **41**:74, 1934.

12. Kassel, V.: Die bösartigen Geschwülste der Schildrüse, Inaug. Dissert., Wurzburg, 1932.

13. Ponhold, H.: München. med. Wchnschr. **18**:692, 1938.

14. Rice, C. O.: Am. J. Cancer **25**:2301, 1931.

As with carcinoma, invasion of the trachea is not uncommon with hemangioendothelial sarcoma. It was present in cases 2 and 6 of Hedinger,[2] in 3 cases (including that reported here) in the material at Bern, and in the cases of Usui,[6] Winnen [7] and Wülfing.[11] De Quervain [15] pointed out the marked tendency toward excessive and atypical bleeding of hemangioendothelioma arising in old adenoma and regards this feature under certain conditions as evidence for a presumptive diagnosis of hemangioendothelioma. The metastases, like the primary tumor, show a tendency toward excessive bleeding, which may be so extensive as to lead to death. Thus, in the case of Blum,[9] death was due to a massive intrapleural hemorrhage from a metastasis of a malignant hemangioendothelioma of the thyroid. In the case reported here death was presumably due to massive hemoptysis from a metastatic intratracheal lesion, and in 1 other case observed in Berne but not reported, death was due to uncontrollable hemorrhage from a metastatic lesion in the mandible at the alveolar process.

REPORT OF A CASE

A 58 year old white man was admitted to the Inselspital (clinic of professor de Quervain) with the following complaints: progressive shortness of breath for over one year, pain in the region of the left shoulder for the past few months, hoarseness for the past two months, difficulty in swallowing for the last several weeks, bloody sputum for the last five days and marked loss of weight. He stated that the "goiter" which he had had for the past thirty-three years had grown considerably in the last year.

History revealed that the patient had never had pneumonia, pleuritis, fever or night sweats. He admitted that he had used external medicaments on the goiter but had never taken anything by mouth. The family history was not relative except for the fact that his brother had a "goiter."

The patient was emaciated, with marked pallor of the skin and mucous membranes and with cyanosis of the lips and hands. The pulse rate was 120 per minute; the blood pressure was 140 systolic and 90 diastolic, and the respiratory rate was 20 per minute, with mild stridor and coarse tracheal rales. The patient was afebrile. A Horner's syndrome was present on the right side and was well developed.

The thyroid gland was markedly enlarged, measuring 16 cm. in width and from 11 to 13 cm. in length. The thyroid was firm in consistency except that the lower middle part felt cystic. The surface was smooth save for occasional irregular areas. The skin was tightly stretched but was freely movable over the gland. Posteriorly the mass was firmly fixed to the organs of the neck. On the right, behind the sternocleidomastoid muscle was an irregular firm mass, measuring 5 to 6 cm., which posteriorly appeared to be continuous with the enlarged thyroid. The lymph nodes of the neck were enlarged and firm. The right superior thyroid artery was enlarged, firm and palpable. The left could not be felt. The trachea was pushed toward the right but was not palpable.

The thorax showed a mild left kyphoscoliosis, and on auscultation the lungs disclosed throughout moist coarse rales.

The rest of the findings on physical examination were essentially negative.

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15. de Quervain, F.: Deutsche med. Wchnschr. **52**:605, 1926.

Initially the blood showed a hemoglobin content of 67 per cent (Sahli), 3,336,000 erythrocytes per cubic millimeter (anisocytosis, anisochromia) and 14,120 leukocytes, with polymorphonuclear leukocytes 82 per cent, lymphocytes 11.5 per cent, monocytes 4.5 per cent and eosinophils 2 per cent. The sputum was almost pure blood. Microscopically the sputum showed innumerable red and white blood cells and a mixed bacterial flora but no tumor cells.

Two days after admission the patient had hemoptysis, producing approximately 1 liter of blood. The hemoglobin content dropped from 67 to 46 per cent, and the patient went into mild circulatory collapse. He was given supportive treatment. On the fourth hospital day, following intravenous and intramuscular injection of a coagulant and intravenous injection of 10 per cent sodium chloride, the patient had a chill. He continued to bleed by mouth and on the fifth hospital day was given a hemostatic intravenously and intramuscularly without any apparent influence on the bleeding. The hemoglobin content fell to 30 per cent, and there were signs of vasomotor collapse. On the sixth hospital day the patient was given a transfusion of 700 cc. of whole blood. He failed to show improvement and died on the next (seventh) hospital day.

Fig. 1.—Gross specimen with the trachea opened posteriorly. Note the invasion directly under the spreader.

Autopsy.—(Prof. W. C. Wegelin.) This examination was made approximately twenty-six hours after death. Only the pertinent observations are described.

The body was that of a well developed but poorly nourished white man aged 58 years and weighing 63 kilograms.

The esophagus, larynx and trachea were filled with bloody fluid. The mucous membranes of the esophagus and larynx were pale. The trachea was markedly compressed on the left and displaced to the right. In the region of the thyroid the mucous membrane of the trachea was replaced by a slightly elevated, irregularly shaped, flat, dark red tumor mass, 2 by 3 cm. in diameter, which was continuous with the left lobe of the thyroid. In the immediately surrounding area multiple flat nodules of dark red tumor, varying from 3 to 5 mm. in diameter, were seen in the mucous membrane of the trachea. These were more marked on the left than on the right and extended up as high as the subglottis. In one of these nodules, which measured 4 mm. in diameter and which lay, somewhat isolated, in the lower anterior part of the trachea, a darker red spot, 1 mm. in diameter, was seen.

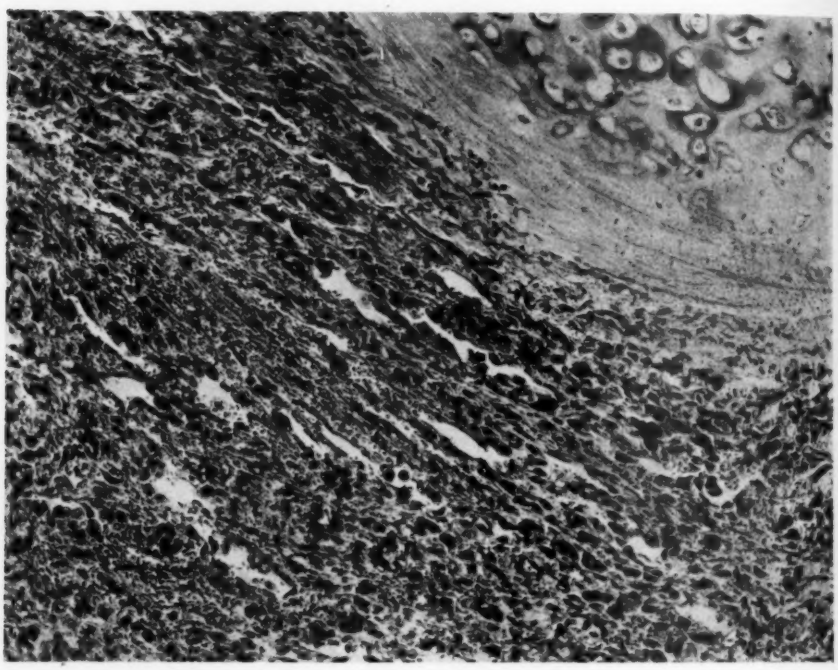

Fig. 2.—Section through the trachea. A portion of the cartilage ring is seen in the upper right hand corner. Note the slitlike spaces, some containing blood cells. Note the tendency to flattening of the tumor cells lining such spaces. Hematoxylin and eosin; × 150.

The left lobe of the thyroid consisted of a firm tumor mass, measuring 11 by 7 by 7 cm. This was firmly and intimately connected to the trachea. The left carotid artery and left jugular vein were patent. No tumor thrombi were present in any of the vessels of the neck. The left lobe of the thyroid was surrounded by a thick, dense connective tissue capsule, 2 mm. in width. The cut surface of this portion of the thyroid showed a large irregular cavity, filled with partially fluid and partially inspissated bloody material. The former was dark red and the latter was chocolate brown and friable. After removal of this material the tumor tissue, which varied from red to grayish red, was seen lining the wall of this

cyst. Within the tumor tissue were innumerable, irregularly distributed slitlike spaces filled with dark red fluid. In some portions of the cyst, just peripheral to the tumor tissue and within the fibrous capsule, a dark brown and somewhat transparent layer of homogeneous material, containing a few slits, was seen. On the tracheal aspect the tumor tissue was found to be directly continuous with the larger tumor mass lying within the trachea.

Anteriorly, extending from the thyroid cartilage to the suprasternal notch, was a roughly ovoid cyst from 10 to 11 cm. in diameter. This cyst, which was attached to the anterior cervical muscles, was filled with a thick, light brown fluid containing a large amount of crumbly, friable material. The cyst was surrounded by a firm wall, from 2 to 3 mm. in thickness, in which was found dark red and brown friable material similar to that seen within the cyst.

The right lobe of the thyroid was also enlarged, measuring 9 by 3 by 3 cm. The cut surface showed many oval or round encapsulated nodules, measuring from 2 to 4 cm. in length and each about 2 cm. in width and thickness. These nodules, which varied from grayish red to brown, were slightly hemorrhagic and transparent. In the upper pole an occasional nodule, gray and measuring about 0.5 to 1 cm. in diameter, was seen between compressed lobules of thyroid tissue.

The cervical lymph nodes on both sides were markedly enlarged, firm and dark red. Many of the nodes showed on the cut surface hemorrhagic areas of softening lying in dark red to grayish red tissue from which bloody fluid could be scraped. The mediastinal lymph nodes were similar.

The other organs were not remarkable from the point of view of this report, and the only reference to them will be included in the final diagnosis.

Histologic Examination.—A section taken through the trachea showed extensive destruction of the entire tracheal wall and mucous membrane. Only the cartilaginous rings were intact. The tumor cells replacing the wall showed a rich variety of shape and arrangement. In general, the cells were roughly polyhedral, with abundant, poorly defined cytoplasmic outlines. The cytoplasm took a pale blue stain, was homogeneous and in some situations was pale bluish red. Many of the cells were phagocytic and contained fragments and whole red blood cells. Other cells were more spindle-like in form, and these were somewhat darker in color. The nuclei of the tumor cells were extremely prominent, with sharply defined nuclear membranes. The nucleoplasm was sparsely granular, and the nuclei contained one or more accentuated dense dark blue nucleoli. Nuclei of irregular size and shape were encountered, and atypical mitotic figures were infrequent. An occasional multinucleated cell was seen.

In many situations the tumor cells lay loosely in a scant fibrillar connective tissue stroma, in which many red and white blood cells were enmeshed. Here the appearance was typically sarcomatous. In other situations were encountered well defined slits, spaces and tubelike structures, lined either by single or by multiple layers of cells. These structures simulated small vessels and capillaries and were lined by robust tumor cells. Many of these spaces contained red blood cells while others were empty. Various degrees of flattening of the tumor cells lining these spaces were noted until vessels indistinguishable from normal capillaries were seen. Mucous glands and mucous membrane were completely destroyed.

Sections taken from the wall of the cystic left lobe of the thyroid showed no recognizable thyroid tissue. The inner surface of the cyst was lined by a pale red amorphous layer of "caoutchouc hyalin," containing no cellular elements but impregnated with granules of hemosiderin. In this layer numerous acicular slits were seen, and the outer portions were slightly calcified. Peripheral to this layer was a dense connective tissue capsule, in which collections of tumor cells

were present. These tumor cells were spindle shape, lay in compact masses and were supported on a fibrillar connective tissue stroma. The cytoplasm was dark and poorly defined, and the nuclei were dense and not particularly prominent. No vascular or slit formation was present here, and the appearance was that of a simple spindle cell sarcoma. In some situations massive hemorrhage was seen in the capsule. Here the tumor cells had phagocytosed hemosiderin and red blood cells. In order to preserve the specimen as nearly intact as possible, no further sections of the tumor mass were made, but Professor Wegelin was convinced that further section of this lobe would show characteristic areas, similar to those seen in the trachea and in the lymph nodes.

A section made from the nodular right lobe of the thyroid showed no tumor tissue, but here a few follicles of glandular tissue with scant colloid still remained in the peripheral portions of the adenomas. A few compressed and atrophic follicles of thyroid tissue were seen in the periphery. The centers of the adenomas were filled with masses of "caoutchouc hyalin," in which were found dilated and cavernous blood spaces, lined by flat endothelium. These cells did not appear to be tumor cells. They may, according to Wegelin,[1] have been either the site of endothelial proliferation as a response to the general tumorous proliferation or the original site of tumor formation.

Sections of mediastinal lymph nodes showed the major portion of these nodes to be replaced by a neoplastic growth composed of robust endothelial-like cells containing prominent nuclei. These cells were arranged in short parallel strands about blood-filled lumens or lay loosely in small clumps. In the latter situation the tumor cells were markedly polymorphic, with large amounts of cytoplasm and with round or oval vesicular nuclei. Here and there occasional spindle cells were encountered. A portion of the tumor tissue was hemorrhagic and was infiltrated by red blood cells and considerable numbers of polymorphonuclear leukocytes. In the sinus of one of the lymph nodes were many endothelial cells filled with phagocytosed hemosiderin.

Anatomic Diagnosis.—The conditions observed were: hemangioendothelial sarcoma of the thyroid, with metastases in the cervical, supraclavicular and mediastinal lymph nodes; perforation of the trachea by the tumor, with implantation metastases in the tracheal mucous membrane; nodular parenchymatous cystic goiter; pulmonary emphysema and edema; hypertrophy of the right ventricle; healed endocarditis of the mitral valve; mild generalized arteriosclerosis; atrophy of the spleen; chronic cholecystitis with cholelithiasis; enlargement of the prostate gland, and hypertrophy of the urinary bladder.

COMMENT

An attempt to determine the exact incidence of hemangioendothelial sarcoma of the thyroid proves difficult for three reasons: In the first place, the incidence of malignant disease of the thyroid varies proportionately with the incidence of endemic goiter and particularly with the incidence of nodular goiter, being greatest in those regions where nodular goiter is endemic. Second, the incidence of this type of tumor in the general population cannot be determined from the study of statistics of surgically removed thyroids or statistics which include the numbers of surgically removed thyroids, for, as Graham[16] has indicated, the incidence of neoplasms of the thyroid gland in surgically

16. Graham, A.: Surg., Gynec. & Obst. **39**:781, 1924.

removed material amounts to a "great exaggeration of the normal incidence." Third, the variegated and manifold histologic picture that the malignant hemangioendothelioma presents makes it appear likely that many tumors of this type involving the thyroid are variously classified by different investigators. It is only by a simultaneous comparison of the incidence of nodular goiters and the incidence of malignant neoplasms of the thyroid and uniform histologic classification of malignant tumors of the thyroid in the same and in a general autopsy population that the true incidence of this type of thyroid neoplasm can be determined. Unfortunately, such correlated statistics are seldom found in the literature, although Wülfing [11] analyzed the material in Freiburg in this fashion. The statistics given by the majority of investigators do not have all these factors in view or include surgical material in the totals. It is beyond the scope of this paper to go into a critical analysis of the incidence of hemangioendothelioma of the thyroid.

Tumors of few other glands offer such a rich variety and complexity of cellular form as the malignant tumors of the thyroid. In one and the same tumor and even in the metastases variations from what appears histologically to be normal thyroid tissue to complex pictures which in the same field simulate both carcinoma and sarcoma are seen. Thus there are not only considerable differences in classification and nomenclature but also divergent opinions as to the relative incidence of sarcoma as compared with carcinoma. Indeed, Ewing [17] and Smith [18] go so far as to question the likelihood of sarcoma ever arising in the thyroid, while Pemberton [19] stated that true sarcoma of the thyroid is rare. In a list of publications given by Kassel,[12] in which the ratio of the incidence of sarcoma to that of carcinoma is quoted, the figures vary from 1:2 to 1:100. The relatively large numbers of instances of sarcoma of the thyroid reported by European workers stand in striking contrast with the occasional one of sarcoma of this organ mentioned by American pathologists. In spite of the fact that Wegelin [1] warned against indiscriminate diagnosis of hemangioendothelioma, he admits that hemangioendothelioma must be considered as one of the more common types of tumors affecting the thyroid. According to Wegelin, many tumors which on first glance have appeared to be sarcoma or carcinoma have on further careful investigation proved to be typical hemangioendothelioma.

On the other hand, he agrees with Ewing [17] and Smith [18] that frequently multiple sections taken from various parts of a tumor which in the original section can be called sarcoma will prove its epithelial origin and nature. However, he is of the opinion [20] that the same factors which produce a great incidence of nodular goiters and malignant tumors in general may be responsible for a greater incidence of sarcoma in his material as compared with thyroids in other parts of the world. It is difficult to conceive why the thyroid should be an exception to the general concept that both sarcoma and carcinoma may

17. Ewing, J.: Neoplastic Diseases, ed. 2, Philadelphia, W. B. Saunders Company, 1922, p. 908.

18. Smith, L. W.: Arch. Path. **10**:524, 1930.

19. Pemberton, J. de J.: Ann. Surg. **87**:369, 1928.

20. Wegelin, C.: Personal communication to the author.

arise in the same organ. The remarkable vascular alteration that attends the growth and development of adenoma frequently is marked by endothelial proliferation of varying degrees, which may well be the starting point of hemangioendothelioma.

There are many features which Hedinger [2] and Wegelin [1] consider macroscopically characteristic and perhaps unique for hemangioendothelial sarcoma of the thyroid. According to them, the tumor is usually solitary and more or less round or oval. It is cystic and filled with fluid or clotted blood. The blood mass is surrounded by a grayish brown transparent layer of so-called "gutta percha hyalin," containing many vascular slits filled with fluid blood. This layer is continuous with the grayish red to pale gray tumor tissue, which often contains small areas of hemorrhage or small yellow foci of necrosis. The layer of hyalin may surround the entire blood mass, but in some cases it may be present in only one portion of the periphery. The outermost portion of the tumor is usually surrounded by a connective tissue capsule, but the tumor frequently breaks through the capsule and invades the adjacent structures.

Microscopically hemangioendothelial sarcoma shows a rich variety of histologic structures. The appearance may resemble that of simple telangiectasia with widening and multiplication of well defined vessels of capillary size. In such cases the endothelial cells are irregular in size and shape and project into the lumens. As further proliferation of the endothelium occurs, the cells become larger, and their nuclei become more rich in chromatin and show mitotic figures. The phagocytic activity of these endothelial cells is marked, especially for red blood cells. In rapidly proliferating areas the endothelial cells become more polymorphic and may lie loosely intermixed with red and white blood cells. In such areas various stages of lumen formation may be seen. When tumor cells proliferate within such lumens and become large and polyhedral, the picture may closely resemble that of carcinoma. Such masses may show secondary lumen formation and become filled with blood elements, and the endothelium then becomes flatter until it resembles normal vascular endothelium. At times the microscopic picture resembles that of sarcoma, with closely packed polymorphic or spindle-shaped cells that may show only a few areas of sprouting or budding and only suggestive evidence of canalization. Occasionally, the microscopic picture may be that of cavernous spaces supported on a rich and sometimes hyalinized connective tissue stroma. These sinuses are lined by robust endothelial cells which occasionally project, like papillae, into the lumen. Limacher [3] described such a case.

Wegelin [1] expressed the opinion that in the majority of cases hemangioendothelioma arises in adenoma rather than from the endothelium of blood vessels in the supporting stroma of an otherwise normal thyroid. In many instances of adenoma showing a layer of "caoutchouc hyalin," widely dilated and thin-walled blood vessels are frequently encountered, in which the endothelium is swollen and occasionally multilayered, and it is in situations such as this that he believes hemangioendothelioma may arise.

Secondary changes in hemangioendothelial sarcoma are frequent. These degenerative changes consist largely of hemorrhage and necrosis.

There is a marked tendency of tumors of this type to destroy the walls and invade the lumens of vessels. Thus, tumor thrombi are mentioned by Limacher [8] and Wegelin.[1] The possibility cannot be excluded that the thrombi are formed from proliferating endothelium of preexisting capillaries which communicate with veins.

The most frequent site of metastasis of hemangioendothelial sarcoma is the lung. Metastases have been described in other situations but are uncommon. Thus, Usui [6] described metastases in the calvarium, tibia and femur; Winnen,[7] in the diaphragmatic pleura; Clivio,[5] in the stomach, liver, sternum and humerus; Matti [8] and Ponhold,[13] in the gums and tongue, and Wegelin,[1] in addition, in the adrenals, heart, mandible, brain and choroid plexus. In about half the cases metastases are found in lymph nodes.

The majority of cases of hemangioendothelioma, according to the material at Bern, occurred in persons between the ages of 45 and 70 years, although the tumor may occur as early as the twentieth year of life. This is also true of carcinoma.

The terminology of these tumors is not always uniform. Wegelin [1] suggested that the term "hemangioendothelioma" should be employed and when used alone should indicate a benign tumor derived from an endothelial proliferation of blood vessels which has a tendency to form vascular channels. He believes that the sarcoma-like forms should be called hemangioendothelioma sarcomatoides only when the appearance suggests strongly that seen in sarcoma; otherwise, he thinks that the term "malignant hemangioendothelioma" characterizes these tumors sufficiently to include those that may in some areas resemble carcinoma. In order to conform with the more usual American terminology, this case has been designated as one of hemangioendothelial sarcoma.

SUMMARY

A case of hemangioendothelial sarcoma of the thyroid, occurring in a 58 year old white man, is reported. The tumor invaded the trachea and metastasized to cervical and mediastinal lymph nodes. The statistical analysis of the relative incidence of this type of tumor is unsatisfactory. Nevertheless, the tumor appears to be more frequent in Europe, particularly in the goitrous regions of Switzerland, than in North America. It probably arises from the vascular structure of an adenoma rather than from that of an otherwise normal thyroid.

Laboratory Methods and Technical Notes

AN INEXPENSIVE EMBEDDING OVEN FOR USE IN ROUTINE WORK ON TISSUES

S. Miles Bouton Jr., M.D., Ingleside, Neb.

Wherever a considerable amount of work on tissues must be done as a part of the general laboratory routine, the recurrent problem of efficient handling and economy of operation presents itself. The average small laboratory does not, in most cases, require complete automatizing and thus usually specializing of the procedure of tissue embedding. More important by far is the general usefulness of every item of equipment placed in the usually limited space at the disposal of the technician.

With these conditions in mind, I attempted a few years ago to design a combination embedding oven and emergency incubator that could be constructed of the simplest and most available materials. Although this laboratory maintains a high standard in the preparation of histologic material, the resulting equipment has given such satisfaction in several years of service that it is thought advisable to describe it here.

DESCRIPTION OF OVEN

The oven is illustrated in figure 1. It consists primarily of a box constructed throughout of 1⅛ inch (2.8 cm.) lumber, thus utilizing the insulating qualities of wood. The door occupies the entire frontage, is completely recessed into the frame of the box and consists of a wood frame holding two panes of glass, separated from each other by an intervening air space. The door is supplied with an inexpensive catch lock. The inside of the box is equipped, a short distance from the bottom, with four regulation screw sockets, two in each side wall, to hold four electric light bulbs (in this laboratory, two 25 watt and two 50 watt bulbs), and the outside, with individual switches, one for each bulb. The electric cord leaves the box at the rear. The top of the box is perforated by six ½ inch (1.3 cm.) holes (the number and diameter of these holes may, of course, vary) within a rectangular space large enough to accommodate a dehydrating jar. This space is marked off by a low wooden frame to hold the jar, and the frame, open at the rear, furnishes guides for a sliding panel which can be adjusted to cover from one to all six holes. For this purpose, the latter are staggered as indicated in figure 2.

The inside of the box is further equipped with rails along both sides, not too close to the bulbs, to hold two removable stout wire mesh trays. A centigrade thermometer is hung on the inside of the door, its mercury bulb insulated against the glass by a piece of very thick cardboard. Thus the temperature within the oven can be read at a glance without opening the door. For purposes of comparison and checking, another thermometer may be attached to the rear wall in such a manner that it also may be read through the closed door.

From the Hastings State Hospital; Juul C. Nielsen, M.D., superintendent.

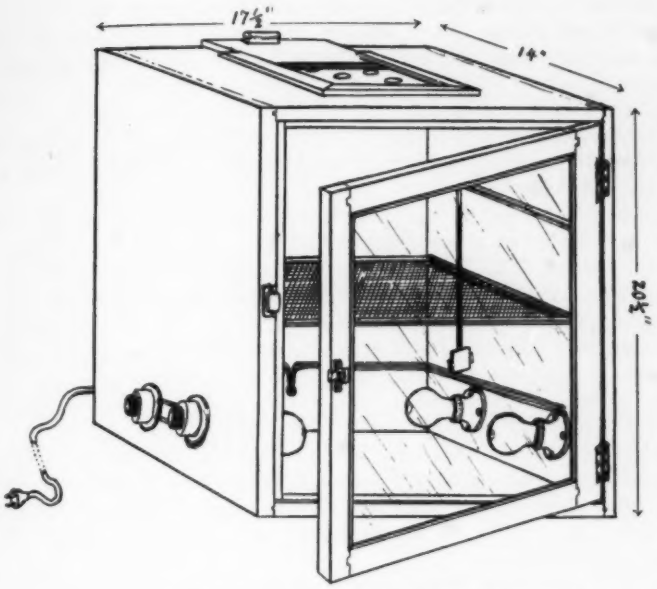

Fig. 1.—Drawing of the embedding oven.

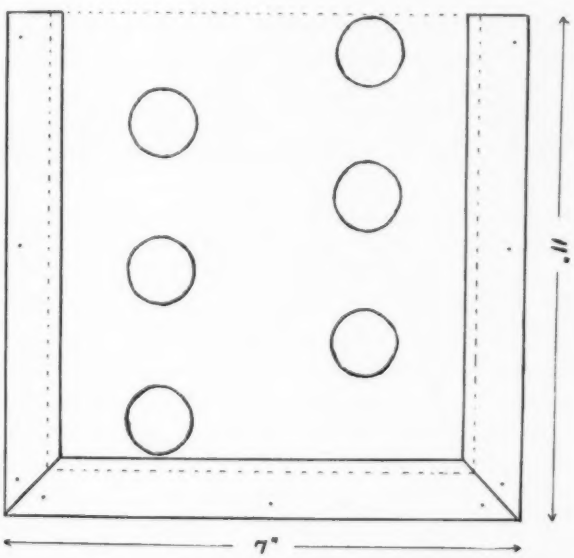

Fig. 2.—Detail: the top of the box, perforated with six holes, staggered to permit covering of from one to all six holes.

Paraffin is kept in metal cups with handles, numbered on the outside, and a mixture of xylene and paraffin (or of dioxane and paraffin) is kept in a glass jar on top of the box for prewarming and saturating the specimens before embedding.

If the box is to be used as an incubator or for any purpose which requires that specimens be kept away from light, the test tubes or other objects may be wrapped in black paper such as that in which x-ray films are shipped. In order to insure a more even temperature on all parts of the specimen, the latter may be placed in a container filled with water.

A wide range of temperature can be achieved with this equipment. With the bulbs now in use, the lowest temperature which I have been able to maintain was a temperature of about 27 C., and the highest, a temperature of about 86 C., the latter without apparent damage to the bulbs or other parts. Once the highest desired temperature has been reached (the rise is gradual), the oven will maintain this temperature on a surprisingly even level, provided the outside temperature (room) does not fluctuate too markedly while the box is in use.

All outside surfaces should be well painted, preferably in white. The approximate dimensions are given in the accompanying illustrations, and are based on the greatest degree of practicability (i. e., ease of getting at all parts of the box, visibility, available space and uniformity of temperature) but may be varied at will. This applies also to the number of light bulbs and trays. The oven described here is the third I have put in use, all three differing somewhat from one another, and is, so far, the most efficient.

The cost of construction, especially in an institution maintaining its own carpenter shop and tinsmith, is obviously extremely low, and the cost of upkeep is negligible, as the bulbs will last a surprisingly long time and use little current.

SUMMARY

A "home-made" embedding oven is described in detail, with its uses.

The chief advantages are the low initial cost, the low cost of upkeep, the great convenience in use and the wide range of usefulness.

The chief disadvantage is the difficulty of maintaining an inside temperature at a constant level if the outside temperature fluctuates too widely.

General Reviews

ELASTIC TISSUE

GEORGE M. HASS, M.D.*
BOSTON

(Concluded from page 365)

PATHOLOGY

The pathologic histology of the elastic fiber may be reviewed briefly in preparation for a general discussion of the alterations which occur in elastic tissue in abnormal bodily states. Atrophy of the elastic fiber is characterized in the early stages by partial or complete loss of specific staining qualities. This refractory stage in case a large fiber is concerned is often preceded by segmentation of the fiber. Subsequently, there is diminution in thickness and ultimate disappearance of the fiber. The assumption of basophilic staining qualities with concomitant loss of affinity for the orcein stain is another common observation. Other evidences of degeneration are skein formation of networks and aggregation of fibers into masses. The individual fibrils may show various thickenings, transverse fractures, granular disintegration, longitudinal fission, vacuolation or fatty degeneration.

In order that there may be some correlation of the pathologic data, the various abnormal states will be grouped under several headings, under which will be treated certain inflammatory, degenerative, neoplastic, vascular, endocrine and congenital diseases, as well as mechanical or chemical factors.

Inflammatory Diseases.—Inflammation is almost always accompanied by deterioration of the elastic tissue. The degree and the rapidity of degeneration vary in different inflammatory processes. A satisfactory explanation for this variability has not been given. Certain general rules, which apply principally to the type of the inflammatory response rather than to its etiologic nature, have been advanced. There are reasons, however, for believing that different etiologic agents may exert different influences. The chief deleterious factors have been attributed to inundation of tissues with alkaline fluids, accumulation of hypothetic toxins, infiltration by inflammatory cells, proliferation of fibroblasts and especially deposition of collagen.

The acute exudative processes usually are characterized by early disappearance of the staining capacity of the elastica, with thinning of the fibrils, followed by almost complete disappearance of networks at

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the height of the process (Unna, 1896). In simple erythema there is no appreciable disturbance of the elastica (MacLeod).

Mechanical crushing does not seriously injure the fibers, but they succumb quickly in the area of inflammatory response (Katsurada). Within six to twenty-four hours after crushing a tissue, Katsurada found slight diminution in the amount of elastica and in the staining capacity of the fibrils. Five days later almost all fibers had disappeared.

The effect of heat has been studied (Unna, 1896). Dry heat caused disappearance of elastic networks as such, but those places where they normally were present elected the specific stains. The regional collagen was either covered by, or had a tendency to imbibe, the residual elastin. Boiling water produced similar changes in the elastica, but no elastin was imbibed by the collagen. Luithlen did not agree with Unna. He contended that burning was not injurious to the elastica.

In bronchopneumonia Sawada failed to find any definite atrophy of elastica. Jores (1902) showed that the apparent diminution of elastic tissue in lobular pneumonia was at least partly dependent on the loss of the normal staining reaction. In chronic pulmonary consolidation Ponfick found the elastica either reduced to a few scattered remnants or invisible.

In erysipelas, phlegmonous infiltration of the cutis and purulent processes the elastic tissue undergoes extensive degeneration. In erysipelas it perishes from colliquation, the early stages being characterized by loss of specific staining qualities and thinning of fibrils, while at the height of the reaction the networks are rarely visible (Unna, 1896). Similar changes have been found in phlegmonous infiltration of the cutis. In these instances segmentation, loss of staining reaction and thinning of fibrils represent successive stages of degeneration (Jores, 1902). In purulent processes there are, in addition to purulent disintegration, segmental degeneration of large fibers and atrophy of delicate fibrils. The elastica may remain in this state of degeneration for a long time (Jores, 1902). B. Fischer described similar changes in the walls of veins which were involved by purulent thrombophlebitis.

Various changes in a few other lesions of exudative nature have been described. The elastica largely disappeared below lesions of dermatitis herpetiformis (White) and pemphigus (Luithlen). Luithlen stated that hemorrhagic lesions and the cutaneous lesions in variola did not affect the elastica. Kreibich could not fully confirm these observations. In contrast to the general rules in inflammation, White found an increase in the elastica of the corium and vessels beneath an ulcer caused by the diphtheria bacillus.

Productive inflammatory processes, especially those of a chronic nature, are usually characterized by the most extreme degree of degeneration of elastic tissue. It is in these lesions that cellular infiltration, fibroblastic proliferation, the formation of granulation tissue and cicatri-

zation exert those effects by which a more or less permanent and complete degeneration or atrophy of the elastica is brought about.

Tuberculosis serves as a classic example of this type of lesion. In the granulation tissue of tuberculosis the elastica degenerates rapidly and extensively (du Mesnil de Rochemont; Krösing and Passarge; Unna, 1896; Guttentag; Lewinsberg, and others). Schmaus found that the primary tubercle is not always depleted of elastica but that the greatest injury occurs in the vascularized granulation tissue. Also there may be a regional zone of atrophy around tuberculous areas, even though the remaining structural elements are normal (Jores, 1902; du Mesnil de Rochemont). In lupus erythematosus, which is questionably of tuberculous origin, Unna (1896) found the elastica unusually normal even where collagen had disintegrated. Melnikow-Raswedenkow described degeneration of the elastica in tuberculosis of the testis; Orth confirmed his findings. Federmann believed that in the early stages of testicular intracanalicular tuberculosis the elastica disappeared first in the walls of the tubules which were involved by the granulation tissue. In the caseous areas Melnikow-Raswedenkow noted that the elastica was not seriously injured. This is in accord with his observations on the good state of preservation of elastica in the necrotic portions of splenic infarcts and with those of Passarge in nonsuppurative gangrene of the skin. Federmann concluded that the more rapid and widespread the caseation necrosis the less was the destruction of elastica. When softening occurred in the caseous areas, the elastica was destroyed rapidly.

The injury to the elastica in tuberculosis is so prominent that this feature has been used in the differential diagnosis between syphilis and tuberculosis. This difference stimulated several authors to postulate a theory of specific elastotoxins. Wechsberg, an advocate of this theory, found that the lesions produced experimentally by emboli of tubercle bacilli in the lungs were characterized after six hours by atrophy of the elastica of the involved vessels. After forty-eight hours there was degeneration of the elastica between the alveoli.

The fate of the degenerated elastic fibrils has been commented on by many authors. Unquestionably, many are destroyed completely. Unna (1896) contended that no new elastic fibers were formed in the cicatrices of tuberculosis but that during cicatrization remnants of elastica might be included in the scar tissue. The possible role of the giant cells is of interest. Ssudakewitsch and Unna (1896) demonstrated remnants of fibers in these cells. Some fibrils stained well and others poorly. Lewinsberg found that there was a direct proportion between the number of giant cells which contained fibrils and the amount of elastica in the tissue. Rona was able to show that calcific clumps in giant cells were calcified fragments of elastica. He assumed that the calcification occurred within giant cells because the extracellular fibers contained no calcium. He

demonstrated also that there was deposition of iron as well as of calcium around the phagocytosed fibrils.

Federmann studied syphilis of the testis. In early interstitial orchitis the elastica in the walls of seminiferous tubules remained intact. So soon as the inflammatory process involved the walls and lumens of tubules the elastic fibers became separated, and eventually they disappeared. The interstitial tissue in fibrous interstitial orchitis contained a few elastic fibrils, which may have been remnants of injured elastica or possibly new-formed fibrils. In contrast to tuberculosis, the fibers around the tubules were retained, even though the lumens of tubules were often completely obliterated. Gummas were similar to the caseous lesions of tuberculosis in that there was preservation of elastic networks until softening or purulent necrosis occurred. At this stage the elastica promptly degenerated.

Krösing described atrophy of elastica in the zones of exudation and cellular infiltration in syphilitic papules. After the exudate had been resorbed, he found that the elastic networks presented an essentially normal appearance. From these observations he concluded that the fibers which had lost their staining qualities during the active phase of the inflammation had regained their affinity for elective stains after the subsidence of the reaction.

Unna (1896) in a study of the primary lesion of syphilis found that at the height of the development of the chancre all elastica had disappeared.

Other diseases which are characterized by the proliferative nature of the inflammatory reaction show rather extensive degeneration of the elastica. By the time that the leprous nodule is well formed, the elastic fibers terminate abruptly at the periphery of the nodule and are absent in the interior. Unna (1896) stated that no new fibrils are formed. Fragments of elastica encrusted with iron and calcium were found by Rona in the giant cells. Fibers also were found in the giant cells in paschachurda (Ssudakewitsch) and cutaneous sarcoid (Hektoen), both being diseases in which the elastica is destroyed. In the granulomatous areas of frambesia (White) and cutaneous blastomycosis (Unna, 1896) the elastica is practically absent.

The less productive inflammatory processes are characterized by deterioration of the elastica, which usually is not as complete or as widespread as in the so-called typical granulomatous lesions. In ulerythema sycosiforme (Sack) and in ulcus serpiginosum (Unna, 1896) there is atrophy of elastica in the areas of cellular infiltration. Unna (1896) stated that in eczema the elastica is destroyed only in areas of massive cellular infiltration. Du Mesnil de Rochemont found that in chronic eczema the atrophy was in the neighbrohood of the areas of great

cellular infiltration or perivasculitis. In dermatitis exfoliativa the elastica is normal except in the areas of cellular reaction (White). White found that in pityriasis rosea there is great reduction in the amount of elastica in the papillary and subpapillary layers of the cutis.

Other conditions which are characterized usually by a slight degree of inflammation may affect the elastica. White found that in Paget's disease of the skin there is atrophy in areas of cellular reaction but hyperplasia adjacent to these areas. In molluscum contagiosum he found diminution or complete absence of elastic fibrils. Jadassohn stated that in striae gravidarum the atrophy is preceded and accompanied by chronic inflammatory cell infiltration. In these areas of infiltration the degeneration of elastica is similar to that which is found in atrophia maculosa cutis. Heuss confirmed these observations and contended that atrophy of elastic tissue may proceed without concomitant degeneration of collagen. Huber described diminution of elastica in the areas of cellular infiltration in atrophia idiopathica diffusa progressiva. There may be thickening of elastic fibers in scleroderma, although partial destruction of the elastica is common (MacLeod). In an instance of bronchial asthma Wawerla found degeneration, calcification and iron impregnation of elastic fibers in the vessels, bronchi and stroma of the lungs. There was a foreign body reaction around degenerated remnants of elastic tissue. Pagel in a study of several cases of bronchial asthma concluded that the only significant alteration in the elastica is an increase which may occur in the walls of medium-sized bronchi.

Cicatrization has been studied by several observers. Unna (1896) believed that granulation tissue becomes free from elastica before it is converted into scar tissue. He contended that elacin and rare elastin fibers may be formed in cutaneous scars. These fibers never attain the systematic architectural arrangement of normal networks. Jores (1900) stated that no elastic tissue is formed in granulation tissue. He found by experiment that many days or months elapsed before elastica had regenerated in appreciable amount in cicatrices. In those scars where there was great sclerosis or interstitial inflammatory reaction the elastica was always absent or sparsely distributed. He was not able to find elastic tissue in keloids. In regard to connective tissue overgrowth, it seems peculiar that in cirrhosis of the liver and in renal cicatrices elastic fibers often have been found where none was present previous to the injury.

It is quite probable that as a result of various lesions the state of preservation or the degree of regeneration of elastica may govern to some extent the nature of the scar. MacLeod stated that if elastic fibers were destroyed in an infectious granuloma, an atrophic scar was the result. He contended that if elastic tissue forms an exoskeleton which supports the collagen, a simple rupture of elastic networks will allow the

collagen to expand, the superficial furrows of the skin will disappear and a smooth scar such as is found in striae and syphilitic rhagades will be the result. It is only fair to add here that much work remains to be done before one may gain an understanding of the peculiarities of cicatrization, not only in regard to certain disease processes but also in relation to the variation among different persons.

Vascular Disease.—Whenever mention is made of pathologic changes of the vascular system, the mind is inclined to dwell on those inevitable changes which accompany and often blight the advancing years. These vascular changes have been divided into two groups, one representing the decay of age and the other the enigma of what some choose to call atherosclerosis. Many consider them as the result of distinct and separate processes. Each may well gain its end with seeming indifference to the other. More often they unite to cut short the span of life.

The effects of age become manifest soon after life has begun. For the most part they pursue a relentless course, retarded in some persons and hastened in others, but still adhering to an established sequence. Early in fetal life the aorta and other arteries show progressive increase in the connective tissue framework. This is accompanied by gradual reduction in the elasticity of the vessel. This process varies in the different arteries as well as in the tunicas of the same artery. It may be studied readily in the intima, where there is gradual increase in the amount of elastic tissue as well as in that of collagen. The increase in age favors the eventual predominance of the latter. During the period from birth to middle life, the original internal elastica lamina in certain larger arteries and in the aorta splits into two or three layers. This increase in elastica soon ceases, and in the senile period the new formed laminae may atrophy and disappear while the collagen is retained. Also, age brings increase in the elastic and collagenous tissue of the media. Similar alterations in the tissue elements of the adventitia are of less significance. Thus, certain changes occur which dispose the vessel walls to become thick and firm. The concomitant loss of elastic tone allows for dilatation, elongation and tortuosity of vessels. These changes are well recognized and may be detected as readily in the retinal arteries as in the aorta. A similar process may progress with remarkable rapidity in such vessels as those of the placenta, where the span of life of arteries is but a few months.

The means by which these changes are brought about have been considered by Wells. He believed that the aging of vital tissues is analogous to the aging of colloidal gels. In both processes there is decrease in hydration with advancing age. This is associated with decrease in elasticity and flexibility. Inasmuch as repeated alterations

in degrees of dispersion of colloidal gels or emulsions diminish their capacity for returning to the original degree of dispersion, Wells expressed the interesting idea that this rule may be applied to the vital elastic colloid, elastin. In support of this is an extreme example: Apparently elastic fibers which have been damaged by overstretching are particularly prone to undergo calcification and iron incrustation.

One of the most common morphologic changes in the elastica of aging vessels is "granular" degeneration. Wells considered this as possibly a late visible stage in the dehydration of elastin, being evidence of aggregation of colloidal micellae. At first this is more prominent in the inner part of the media. Gradually it progresses toward the adventitia. When the degeneration is great, the granules collect in small clumps between the lamellae. The large lamellae of elastica are unequally swollen and show areas that stain poorly with orcein. These parts atrophy so that the concentric bands appear to be discontinuous. Some fibers show the elacin reaction. Schultz was able to follow the progressive change of elastin into elacin, the process beginning in childhood and increasing with age. Granular degeneration and diminished affinity for elective stains are found commonly in the inner sheaths of the internal elastic laminae of small and medium-sized arteries. Ribbert, Reich and Jores (1902) found a definite relation between granular degeneration of elastica and fatty deposits in the intima.

There is a tendency for aging or overstretched elastic fibers to imbibe or absorb substances of low solubility. Because of this property, the fibers become impregnated or coated with such materials as calcium, iron and silica. There is no apparent uniform proportion between the amounts of each which are deposited. The microscopic structure of the calcification often shows a radiating or concentric arrangement, which supports the theory that the deposits are formed under the rules which govern the deposition of crystalloids in colloidal matrices under the influence of colloidal forces (Rusznyak).

It has been said that normal arteries contain a small quantity of calcareous material (Ribbert; Klotz; Faber). This can be demonstrated in the first years of life and occasionally before birth. Later in life it occurs chiefly in the elastic lamellae of the media of elastic arteries. Also, calcification of the internal elastic membrane is common. Here calcium granules may be found either in or alongside the fibrils, which are often undulate or fragmented. Ameseder found that in the human aorta decrease in elastin is proportional to increase in inorganic substances.

Ravault (1929) studied calcification of arteries by the method of microincineration. He found calcific deposits in the media of aortas that were histologically normal. He believed that the calcium was

associated with elastic elements and occasionally was incorporated in the elastic substance. Furthermore, he stated that the media adjacent to the calcified intima of diseased vessels is often uncalcified and shows less than the expected normal content of ash. In the Mönckeberg type of medial calcinosis he found the mineral deposits in and around the collagenous and elastic elements rather than primarily in muscle fibers.

Atherosclerosis, according to most authorities today, is a process that is fundamentally distinct from the process which is discussed in the preceding pages (Aschoff). Although degeneration of the elastica often may precede or accompany the deposition of atheromatous substances, the two processes fail to exhibit any impressive parallelism. In fact, Anitschkow stated that in experimental cholesterol atherosclerosis of rabbits the elastic lamellae act as barriers to the infiltrating lipoids and that there is no fatty infiltration of the elastic laminae such as may be found in man.

Other pathologic states which may involve the elastica are accumulations of so-called "mucin," "hyalin" or "amyloid" in the vascular wall. The extent to which degeneration of elastica is dependent on these substances is unknown. There is no incontrovertible proof that one process is intimately allied with the other. Schultz demonstrated "mucin" in arterial walls. He believed that it had an affinity for fat and calcium. The deposition of hyalin and amyloid may be so extensive that elastica cannot be demonstrated. In these instances it has degenerated completely, has lost its elective staining qualities or has become obscured.

The effects of mechanical injury to the elastica of vessels and the result of such injury have received careful study. Jores (1900) described a new formation of elastica in the intima of vessels within eight to ten days after ligation. This was well developed within twenty days. Later (1902) he was able to show that after traumatic injury to the vessel wall abundant regeneration of elastica occurs. In this regard it may be said that new-formed vessels eventually may gain a full complement of elastic networks and that formation of elastic tissue in organizing thrombi is of common occurrence. Petroff and Glasunow found that lesions caused by pinching or cauterization of arteries lead to increased permeability of the arterial wall. The subsequent changes in arteries of the elastic type depend on the degree to which the elastic framework of the vascular wall has been injured (Ssolowjew 1929). Great injury results in replacement by granulation tissue, which arises at first in the adventitia. If all elements except the elastica are injured, there is regenerative proliferation of the cellular parts of the uninjured media. In this repair new elastic fibers may be formed.

The role of the elastic networks in the formation of aneurysms was discussed at great length by early authors. Von Recklinghausen, Helm-

städter, Manchot and Eppinger believed that the basic factor leading to the development of the aneurysm is a rupture of elastic fibers. They contended that the sharp interruption of the continuity of the elastic lamellae favored the thesis that the rupture is of mechanical rather than of inflammatory origin. Koester, Krafft and Znurdeeg considered these ruptures as secondary to inflammatory processes.

Thoma emphasized the significance of altered elastica in the pathogenesis of arteriosclerosis. He directed attention to small fissures or defects in the media. These seemed to be independent of the state of preservation of the regional elastica. He believed that in addition to the disruption of the elastica there is an involvement of smooth muscle cells and connective tissue. He commented on the presence of cicatrices and leukocytes in the region of tears and noted that degeneration of the medial elastica usually is found only where there is a fairly cellular overgrowth of connective tissue. Ssolowjew (1930, 1932) produced isolated tears in the elastic lamellae of the inner layers of the media by mere displacement of the carotid artery. The tears appeared after a lapse of time. The process of repair eventually was completed by replacement of the tears chiefly by intimal plaques that contained elastic elements.

If one assumes that the rules for elastic colloids in vitro are applicable to elastic tissue, repetition of extension, even within physiologic limits, soon will reduce the elasticity of this tissue and increase its elastic resistance. Apparently elastic fibers are damaged by overstretching, and it is said that fibers so treated are prone to bind calcium and iron. This may be of interest in relation to hypertension. It has been known for a long time that chronic hypertension and reduplication of elastic laminae in the intima of renal arteries usually occur together. A similar, although somewhat different, picture is found in the compensatory adjustment of an artery to a decreased blood flow. In this instance, loose collagen and elastica symmetrically thicken the intima and thereby reduce the size of the lumen. The several types of arteritis of specific or nonspecific nature show various degrees of deterioration and reparative regeneration of the elastica. These usually follow the rules which govern the general changes in inflammatory conditions. They have been considered elsewhere and need no repetition.

Thromboangiitis obliterans, because of its peculiar character, may be considered in more detail. Similar pathologic changes may occur in Raynaud's disease, arteriosclerosis and scleroderma. Krompecher (1930) believed that the stenosis of the lumens of certain vessels was produced primarily by proliferation of specific cells, "the elastoblasts." He demonstrated a delicate elastic membrane around each elastoblast. He con-

tended (1931 [*c*])that formation of elastica occurs only if these cells are present and that these cells degenerate as soon as the elastic elements are fully formed. The same process may be repeated in such a way as to cause stenosis of the lumens of the vessels which form in thrombi. Jäger made a careful study of thromboangiitis obliterans. He found that during the acute stages the elastica of the vessels was chiefly unstainable. In later stages the elastica often showed variable changes. The external elastic membrane frequently was disrupted and calcified. There was rarely a foreign body reaction to the remnants of elastic fibers. The adventitia usually showed an increase in the elastica. The chief findings in small vessels were folding of the internal elastic membrane, intimal thickening and in the later stages increase in the elastic fibers of the intima.

Although not of inflammatory origin, the alterations which occur in the ovarian and uterine arteries under various physiologic stimuli are of unusual interest. Woltke, Kon and Karaki and Böshagen described medial degeneration and endarterial formation of new vessels in the arteries around ovarian follicles. Eden and Lockyer described a similar process in uterine arteries. The peculiar changes which have been designated as ovulation and menstruation sclerosis were studied carefully by Sohma. Before the establishment of menstruation the cortical arteries of the ovaries are devoid of elastic tissue except for a single internal elastic membrane. With the beginning of the menstrual periods an elastic network appears in the muscularis and often condenses to form an elastica externa. During menstruation there is distention of arteries. Serous exudate permeates the walls, and fibers rupture. After menstruation a network of elastic fibers reforms in the media. Similar changes occur in vessels around a ripening follicle. After rupture of the follicles there is great increase in the medial elastica, and the muscle is replaced by a fibroelastoid substance. Beneath the vascular endothelium a new media appears. This displaces the degenerated media toward the periphery. This process may be repeated during several ovulations so that the elastic remnants of two or three medial layers which have degenerated in succession may be found encircling the vessel.

Similar degeneration and reformation occur in uterine arteries on termination of pregnancy. At the menopause the arteries of the uterus and ovaries show atrophy of the peripheral medial remnants and of the internal well formed media. During this involutional period senile arteriosclerotic changes commonly are superimposed on the physiologic retrogression.

The rapid involutional changes which occur in the placental arteries and the ductus arteriosus are somewhat analogous but not identical to those which are described in the foregoing paragraph.

Little seems to be known about lesions of the elastica which may be produced by toxins or those which may occur in "allergic" responses. The deleterious effects of diphtheria toxin on the walls of blood vessels are well known. Many authors have contended that epinephrine is injurious to the vessels. The sclerosis induced in rabbits by experimental administration of epinephrine occasionally is characterized by stretching and disintegration of the elastica. These changes are late manifestations and follow the deposition of calcium granules in the ground substance. There may be new formation of intimal elastica. These are not specific changes because many substances produce alterations similar to those observed in epinephrine-induced sclerosis (Anitschkow).

The elastica of veins may be affected in many ways. The changes occurring with advance in age are similar to those which occur in arteries, but there is less hyperplasia of the elastica. Increase in collagen commonly occurs throughout the wall, but atheromatous and calcific deposits are rare. The most important changes are those which result from stasis of blood. In such instances there is primarily degeneration of smooth muscle cells and of elastic fibers in the media. New-formed elastic and collagenous fibrils commonly appear in the intima adjacent to the areas of degeneration and fibrosis in the media (Scagliosi). In acute phlebitis and purulent thrombophlebitis widespread degeneration and disappearance of elastica often occur (Fischer).

Degenerative Diseases.—This is not a satisfactory subgroup, but it includes certain conditions which are characterized by atrophy and degeneration of the elastica, independent for the most part of mechanical or inflammatory influences. Among these conditions certain cutaneous diseases, especially those which accompany advanced age and certain degenerations of blood vessels, have received the most study.

Senile atrophy of the skin is accompanied by important alterations in the elastic networks (Schmidt). In the skin of the face the fibrils become swollen. They undergo granular degeneration and gather in hyaline masses. The swelling of the fibrils is either diffuse or in the form of numerous varicose thickenings so spaced as to suggest a rosary. The fibrils may either retain their specific staining reaction or develop an affinity for dyes that are rejected by normal fibers. In the second form of degeneration the fibrils disintegrate into granules which are highly refractile and which retain the elective staining qualities. This type of deterioration finally may lead to formation of strands or irregular clumps of granules, which tend to coalesce so as form homogeneous masses. Schmidt believed that in this way hyaline masses which have an affinity for the elastic stains are formed. These may become so numerous that in certain severe cases structureless homogenenous masses replace a large

part of the upper cutis and in rare instances may be distributed throughout the entire cutis. Reizenstein, Krösing and Passarge and Krzysztalowicz, however, contended that the strands and clumps are comprised of a maze of very delicate fibrils rather than of conglomerate masses of fused granules.

Unna (1896) found that some of the fibers in senile skin which stain weakly with orcein have an affinity for basic aniline dyes. He believed that there was some chemical alteration of the substance of the fibrils. He named the changed substance elacin to distinguish it from elastin of normal tissue elements. The substance elacin is found especially in senile skin and in other conditions in which atrophy is prominent. In addition to alterations in the elastica, he described changes in the collagen of senile skin. The substance of fiber structures which have the physical appearance of collagen but the staining qualities of elastin, he named *Kollastin*. The substance of collagenous fibrils which have the staining reaction of elacin was designated by him as *Kollacin*. In colloid degeneration of the skin he believed that the "colloid" is formed from *Kollastin*, and that with its appearance the specific elastin stain is lost.

Reizenstein found that the changes described in the elastica of senile skin are not restricted to the skin of old people but are detectable in persons as young as 26 to 28 years of age. Unna (1896) confirmed this and concluded that the "senile" type of degeneration of elastica is less dependent on age than on climatic influences. Krzystalowicz agreed with Unna. He described elacin in the upper layers of the cutis in young persons and in the deeper layers in older persons. *Kollastin* appeared in the upper layers of the skin of young persons, while *Kollacin* formed in larger amounts in aged persons. He noted that the various changes were more prominent in men than in women, especially among the laboring classes. Furthermore, the exposed parts of the body occasionally were the only areas which showed changes.

Himmel emphasized the occurrence of areas of cellular infiltration in the skin. He believed that the "senile" changes in the elastica might be attributed to inflammatory reactions to unknown deleterious agents.

There are a few other cutaneous lesions in which the elastic tissue shows changes that may be considered degenerative. In striae gravidarum and striae distensae the beginning of the deterioration of the elastica is associated with chronic infiltration of the tissues by inflammatory cells. This fact does not exclude entirely the possible importance of hereditary, mechanical or endocrine factors. These will be discussed elsewhere. Jadassohn found that the degeneration of elastic tissue in atrophia maculosa cutis is similar to that which occurs in striae gravidarum. Huber described significant diminution of elastica in atrophia idiopathica diffusa progressiva. This occurs chiefly in areas

of cellular infiltration in the subpapillary zone of the cutis. The atrophy of elastica which may result from prolonged edema and that which may occur in scleroderma will be considered elsewhere. White found slight diminution of elastica tissue in the deep layers of the corium in cutaneous amyloidosis. In pingueculae Fuchs described skeins and clusters in which the elastic fibrils were so delicate that the aggregates resembled homogeneous masses. Here there was definite degeneration of networks.

An important group of cutaneous lesions, described as xanthomatous in nature, are characterized by degeneration of the elastica, with formation of clumps and skeins of fibrils. Darier segregated a group in which there were similar pathologic changes. He gave this entity the name "pseudoxanthoma elasticum." Since his original description other names such as "elastoma" and "elastic naevus" have been applied to the condition. This disease usually is found in young people and often is familial. The primary lesions tend to be papular and symmetrically distributed. They appear chiefly in the skin of the unexposed parts of the body, especially in the regions of articular folds. Here, the skin may become greatly pendulous. Darier described an increase in the elastic substance of the skin. Many fibers were thickened, basophilic, vacuolate, nodular or ruptured. Sometimes they were in clusters or in the form of scattered granules. Throne and Goodman reviewed the literature carefully. They found that the elastica in the middle and deep portions of the corium showed the greatest degeneration. This was exhibited chiefly by swelling, proliferation and fragmentation of fibers. The collagen was either normal or swollen and basophilic. Giant cells in which fragments of elastica were found were occasionally present. Calcification rarely occurred. Sternberg did not believe that the masses of "elastica" in pseudoxanthoma elasticum were of elastic tissue region. Grönblad in an excellent monograph emphasized the high incidence of "angioid streaks" of the retina in the disease. He attributed their formation to degeneration of the choroidal elastica and contended that the syndrome was a manifestation of a systemic disease of elastic tissue, affecting chiefly the skin, buccal mucosa and heart. Another site of predilection was the choroid, by the involvement of which angioid streaks of the retina were formed. Apparently the blood vessels were affected to a lesser extent.

Jones and his collaborators did not believe that the disease could be differentiated histologically from senile elastosis. They contended that the disorder should be considered as a peculiar manifestation of early cutaneous changes similar to those in senile skin. Lewis and Clayton also found that the processes were similar.[6] Montgomery contended that in typical cases the degeneration seemed to be almost entirely confined to the elastica and that one should be able to differen-

tiate the disease from senile elastosis. He expressed the belief that the disease was the result of defective formation of elastic tissue.

A process closely akin to that of pseudoxanthoma elasticum has been described by Juliusberg. In this disease there were yellowish cutaneous lesions which resembled scars. In these areas the alterations in the elastica were similar to those in pseudoxanthoma elasticum.

In retrospect, the evaluation of certain studies of cutaneous elastica should be guided by a thorough knowledge of the normal variations in elastic tissue. Awoki found in grossly normal skin from routine autopsy material that elastic tissue showed wide variations which were within the limits of many "pathologic" changes attributed by other authors to the effects of disease.

The degeneration of the elastica which accompanies advanced age may be found not only in the skin but also in other parts of the body. In the spleen the elastica may undergo atrophy, which is especially prominent in the inner half of the capsule. Diminution of elastica is common in the sclera of aged people (Jaensch). There is a question as to the part played by atrophy of elastica in the senile type of non-obstructive pulmonary emphysema. It is well known that deterioration of the elastic tissue in arteries is an accompaniment of advance in age. These changes will be considered in greater detail elsewhere.

Mechanical Factors.—The alterations in elastica which generally are assumed to be the result of mechanical forces should not be considered as wholly secondary to physical trauma, because often in these instances there is an associated inflammatory reaction. Neither do all observers believe that the deterioration of elastica in purely inflammatory lesions is of chemical origin. Du Mesnil de Rochemont contended that the injuries were produced by chemical influences. Guttentag and Schulz believed that the changes in the elastica in all lesions, inflammatory or otherwise, are a manifestation of pressure atrophy, such as Obermuller described in instances of prolapse of the vagina and prolonged use of a pessary. These conflicting opinions have been carried into the discussions which deal with the various conditions in which there is injury of the elastica.

Thus, in relation to the vascular system, the question as to whether the disruption of elastica fibers in arterial walls is always of mechanical or inflammatory origin has been studied. Von Recklinghausen found fissures in the media of fresh miliary aneurysms. Helmstädter, Manchot and Eppinger advanced the theory that a mechanical rupture of the elastic fibers is the basic factor which leads to the formation of an aneurysm. It is well known that in many places where the arterial elastic tissue has been destroyed the constituent elements of the media have been replaced by fibrous tissue. Koester, Krafft and Znurdeeg

contended that these foci are evidence of an inflammatory process which involves all types of structures. Manchot, Thoma, Zwingmann, Schulmann and Eberhardt believed that the effects were produced fundamentally by mechanical means. Manchot demonstrated abnormal elastica at a distance from the scarred areas. Thoma emphasized the significance of altered elastica as of importance in the pathogenesis of arteriosclerosis. He found small fissures or defects in the media. These appeared to be independent of the condition of the regional elastic tissue. He believed that all elements of the arterial wall frequently are involved and that often in the region of tears in the elastic lamellae there are cicatrices and leukocytes. If only a few elastic fibers parted, it seemed that the connective tissue and smooth muscle, even though not directly involved, tended to undergo atrophy and hyaline degeneration. Jores (1902) stated that although elastic fibers are destroyed by inflammation, a traumatic injury to the wall of a blood vessel evokes abundant regeneration of elastica. Fabris, after producing necrosis of the walls of blood vessels concluded that the destruction of elastica was similar to that which previously had been considered to be of mechanical origin. Katsurada came to the conclusion that pinching the skin causes no significant changes in the elastica unless there is an inflammatory reaction.

The pathogenesis of cutaneous striae is not entirely clear. The factors which suggest a mechanical origin are as follows: the occurrence of the striae with a rapid increase in body volume, their appearance along lines of cleavage of the skin transverse to the planes of greatest tension, their development in those sites where the skin is delicate, and finally, the interpretation of the histologic changes. Brünauer found certain exceptions to the mechanical theory. The exceptions indicated that toxic or endocrine influences may play a role. The theory that there may be specific "elastotoxins" was entertained.

In early hypertrophic striae or "welts," an edema of collagen, an increase in the number and size of the nuclei of connective tissue cells, dilatation of vessels and infiltration of perivascular tissues with round cells and polymorphonuclear leukocytes are common (Ebert). The normal elastica in such striae is supplanted by a multitude of delicate, poorly-stained fibrillae arranged parallel to the surface of the skin. There is some collacin. The elastica in the atrophic striae tends to disappear, the connective tissue condenses, the inflammatory reaction subsides, and new-formed elastic fibrils may arise at the margins of the lesion.

Other instances in which abnormal stresses may affect the elastica are pulmonary stasis, emphysema of the lungs, asthma, uterine pregnancy, phlebectasia and edema. In chronic pulmonary congestion there

usually is great diminution in the amount of elastica. Jores (1902) showed that this was more apparent than real, because many fibers which remained were not readily distinguishable; they simply had lost their affinity for specific stains. Degeneration of elastic tissue and diminished elasticity of the lungs have been proposed as causes of nonobstructive emphysema of the lungs. Sudsuki found that in the nonemphysematous portions of such lungs the elastica was normal. In the emphysematous portions there were variable degrees of change. The larger fibers in the early stages of the disease seemed to exhibit the greatest degeneration. Later the delicate fibrils were involved. The deterioration of the elastica occurred no earlier than recognizable degeneration of other tissue elements. He concluded that emphysema is not caused by either deficiency or degeneration of the elastic networks. Loeschcke described hyperplasia and Orsos (1906) found evidence of regeneration of elastica in emphysematous lungs. Wawerla described widespread degeneration, calcification and iron impregnation of elastic fibers in the lungs of a patient with bronchial asthma. Pagel stated that the only alteration in bronchial asthma is an increase in the amount of elastica in medium-sized bronchi.

There are interesting changes in the pregnant uterus. Woltke, W. Pick and Iwanoff showed that there is great increase in the amount of elastica during the early stages of pregnancy. In the second half of the gestation period the fibers undergo atrophy in a manner similar to that which occurs in pathologic processes. At the end of pregnancy the diminution is so great that Davidoff and Poroschin offered this as an explanation for certain ruptures of the uterus.

Scagliosi studied the effects of stasis on veins. He concluded that the degenerative changes in the medial elastica are secondary to the degeneration of smooth muscle. He described new formation of intimal elastica adjacent to areas of medial degeneration.

Most authors agree that prolonged edema is very injurious to the elastica. The gradual disappearance of the networks in the edematous skin affects the delicate fibrils first. The degeneration progresses inward from the papillary zone and is most severe in those tissues which have the richest complement of elastica (Unna, 1896).

Little is known about the atrophy of elastica from disuse except so far as this factor may exert an influence in the pathologic states which have been considered. In this regard, however, Loeschcke described an atelectatic accessory pulmonary lobe in which the elastica had failed to develop beyond the stage found in the infant at maturity.

Of further interest are the studies on skin grafts made by Enderlen. In Thiersch grafts the elastic fibers gradually degenerate. New elastic networks develop. The new-formed fibrils appear within twenty-one

to thirty days. Fifteen to eighteen months later, the tissue again is supplied richly with elastica. The new elastic tissue does not have as orderly an arrangement as the networks of normal skin. The elastica completely degenerates in the Krause grafts, and no regeneration occurs.

Chemical Factors.—It would be presumptuous indeed to dwell at great length on alterations in the chemical environment and their possible effect on the functional integrity or morphologic aspects of elastic fibers. In the preceding pages allusions have been made to a few of the facts which are established. The atrophy and degeneration which occur locally in various inflammatory lesions and in neoplasms have been ascribed by certain authors to chemical influences. The general diminution in the functional efficiency or actual deterioration of elastic fibers in certain systemic diseases has been attributed to "toxins" for want of more accurate knowledge. In experimental vitamin C deficiency Wilton showed that there is atrophy of elastic fibers and change of elastin into elacin, thus continuing the original observations made by Rheindorf bei Tuschler. It has been learned that applications of arsenic, tar and roentgen rays to the skin of mice within certain limits stimulate local development of "resorcin" fibers (Bierich, 1922). The influence of endocrine secretions and constitutional and hereditary factors will be considered later.

Thus, although the chemical influences and the means by which they may alter the elastica are wrapped in obscurity, one knows a little more about the affinity of this tissue for certain metallic elements, namely, calcium, iron, silica, silver and lead.

The affinity of elastic tissue for calcium and iron has been recognized for a long time. These two elements may be deposited in or around the fiber. Incrustation apparently is more common than impregnation. The two elements may occur together or independently, and there is no absolute ratio between the amounts of each which may be deposited (Gierke). An injured fiber has more affinity for these metals than a normal one. However, Katase produced calcium deposits in apparently normal fibers by injecting large quantities of calcium into animals. Learner obtained similar results with an overdosage of parathyroid.[1] Köckel, Bittrolf and Davidsohn were among the first to demonstrate calcified and iron-incrusted fibers in the lungs. Hofmeister stated that calcification of the elastica in the lungs usually accompanied *Kalkmetastase*. Jores (1902) found that calcium often is deposited in and around elastic fibers in blood vessels. The elastica interna is involved most frequently. Ravault studied this distribution by microincineration. Ehrlich discussed fully the iron and calcium incrustation of elastic fibers, especially that in the cerebral and thyroid arteries and

1. The preparation used was equivalent to solution of parathyroid U. S. P. XI.

in old splenic infarcts. Schuppisser described several instances in which the elastic fibers had attracted large amounts of iron. Lubarsch stated that in one type of calcium and iron deposition in the spleen the distribution corresponded to that of the elastica. Davis and Warren described calcification of the elastica of the skin in diabetes. The occurrence of fibrils incrusted with calcium and iron within giant cells was described by Rona. Pelagatti and Sprecher did not accept these as elastic fibers. Rona assumed that the deposition of metals occurred after the fiber had been engulfed by the giant cell. He demonstrated these fibers in giant cells of tuberculosis and leprosy. Similar inclusions in giant cells were found in mycosis fungoides (Delbanco), in pascha-churda (Ssudakewitsch) and in sarcoid-like lesions (Hektoen).

Sobotta stated that elastic cartilage undergoes calcification much less frequently than hyaline cartilage.

According to Wells, the aging of elastica enhances its affinity for silica as well as its affinity for calcium and iron.

The attraction of elastic tissue for silver was noted by Blaschko, who studied instances in which silver particles had become embedded in the skin of silver workers. He found that the silver became dissolved in the course of time and then precipitated largely on the regional elastica. In a case of silver poisoning by the alimentary route, it was only with great difficulty that he was able to demonstrate deposition in the elastic fibrils of the skin. Unna (1896) stated that silver deposits in the skin always were limited to the connective tissue, especially to the elastic fibrils and limiting membranes. Lubarsch cited an instance of argyrosis in which silver deposits in the spleen were limited to the elements of connective tissue. So far as lead poisoning is concerned, it has been stated that there is a tendency toward severe arteriosclerosis. Injections of large amounts of lead fail to disclose any specific affinity of the elastic tissue for lead (Kumita).

Tumors.—Tumors, especially if they are malignant, are usually entirely free from elastica. Elastic fibers may occur in the stroma, but they are few and usually represent the elastica of the invaded tissue which has escaped destruction by the neoplasm. Krösing and Passarge found disintegration of delicate fibrils at the margins of tumors. This has been attributed by many subsequent observers to the proliferation of connective tissue and the general inflammatory reaction. Kromayer described degeneration of fibrils in the area of cellular infiltration in the stroma of tumors. In neoplasms experimentally induced with tar, nematodes and roentgen rays similar alterations occur in the elastica (Bierich, 1922). In certain instances the "elastic" fibers in the cutis were increased, but in these cases the epithelium had not extended into the corium. As the epithelial cells invaded the corium, there was disappearance of elastica, especially in the zones adjacent to the tumor.

Various types of atrophy and degeneration of elastica may occur in association with neoplasms. The most common are simple atrophy, skein formation and clumping of fibrils. Elacin fibers also are frequent (White).

Several benign epithelial tumors have been studied for elastica. White found an abundance of elacin fibrils but no elastin fibers in senile keratoses. There are nodular accumulations and dense networks of elastica in epithelioma adenoides cysticum (W. Pick). Unna (1896) found that in epithelial cutaneous nevi, if cell nests are prominent, there is a tendency for elastica to disappear between them. He believed that this loss of elastica aided in the mechanism by which the nevus became elevated above the plane of the skin. Most interesting of all is the carcinoid or argentaffin cell tumor, in which the stroma is rich in elastic fibers, smooth muscle and nerve fibers (Masson). Bailey studied benign and metastasizing tumors of this type. Elastic tissue was abundant and comprised an integral part of the stroma, not only in the intraintestinal growths but also in the mesenteric extensions and metastases in lymph nodes. He found hyperplasia of the elastica of the adventitia of small and medium-sized arteries. The elastic fibers of the tumor seemed to be derived in part from the networks in the vascular walls. He concluded that the formation of elastic tissue fibers in considerable numbers as a part of the stroma seemed to be a unique property of argentaffinomas.

Benign tumors of mesenchymal origin are little more favorable to the development of elastic fibers than benign epithelial growths. Virchow (1858, 1889) cited instances in which new-formed elastic fibers were demonstrated in cavernoma, enchondroma, endothelioma and myxoma. Karrenstein described elastic fibrils in myxoma of the heart and mentioned several similar tumors that he collected from the literature. Unna (1896) stated that there is little or no elastica in cutaneous fibroma. He found absence of elastic tissue in angioma and believed that the lack of elastica in the hypertrophied venous channels of the angiomatous nevus offered an important criterion by which the vessels could be distinguished from normal veins. White noted that elastic tissue is scanty in cutaneous leiomyoma. On the contrary, elastic networks frequently occur in uterine leiomyoma.

Very few studies of elastic tissue in malignant tumors have been reported. Mention has been made concerning malignant carcinoid or argentaffinoma of the alimentary tract. It is well known that carcinoma of the skin quickly destroys elastic tissue. Zieler concluded that the degeneration occurred in the zone of infiltration at the periphery of the tumor. He found that the destruction of the elastica

is greater if the zone of infiltration is advancing rapidly. Degeneration of elastic tissue without evidence of regeneration occurs in gastric carcinoma (Inouye). New formation of elastic tissue rarely has been described in the stroma of cutaneous and mammary carcinoma (Virchow, 1858). White found that the elastica was either reduced or absent in cutaneous sarcoma. Virchow (1858) cited instances in which there was apparent new formation of elastic fibrils in fibrosarcoma. The areas of infiltration of the skin in mycosis fungoides show either great reduction or complete disappearance of elastic networks (Jores, 1902; White). Delbanco found inclusions which were presumed to be fragments of elastic fibrils in the giant cells of mycosis fungoides. In this regard it may be added that von Hansemann and Zieler described fragments of elastica in the cytoplasm of various types of tumor cells.

So far as I am aware there is no tumor which is characterized principally by formation of elastic tissue. It seems that such tumors must occur, because the elastic fiber as to its origin has many features in common with the collagen fiber. Tumors which form an abundance of collagen are frequent. It has been suggested that pseudoxanthoma elasticum is essentially neoplastic, but there is no good reason for supporting this contention.

Congenital, Hereditary and Endocrine Diseases.—The influence of the endocrine glands on the formation and maintenance of the bodily tissues often is bound intimately with what one may choose to call constitutional, congenital and hereditary states. Therefore it seemed reasonable to consider these subjects at the same time.

Most authorities believe that the disease pseudoxanthoma elasticum is a manifestation of hereditarily defective elastic tissue. This malady, which is characterized principally by a peculiar degeneration of the cutaneous elastica and by angioid streaks in the retina, has been discussed briefly in the preceding pages.

Epidermolysis bullosa hereditaria is a rare congenital disease in which vesicles and bullae form in any part of the integument or mucous membranes subjected to traumatism. In this disease Engman and Mook described absence of elastic tissue in the papillary and subpapillary regions of the grossly normal derma. The elastic tissue in the deeper layers of the cutis was scanty and structurally defective. Other workers have corroborated their observations. This abnormality of the skin has the hereditary transmissibility characteristic of a mendelian dominant, affecting both sexes in almost equal numbers.

The constitutional bodily type designated as the asthenic habitus is due, according to Payr, to a low value of contractile and elastic elements. He claimed that patients of this type have an increased tendency toward overgrowth of connective tissue in the process of repair. No careful

comparative histologic studies have been made of the tissues of various constitutional types, but if such studies are ever made, the results should be of interest (Bauer). Sudsuki stated that there is wide variation in the amount of elastica in the lungs of normal people. He made no attempt to correlate his observations.

If elastic tissue has a part in the maintenance of elastic tone in the skin, it is reasonable to assume that among various persons there may be not only quantitative but also qualitative differences of this tissue. In this regard the "india rubber men," classic sideshow attractions, may be mentioned. These persons have abnormally extensible skin, which is of such a nature that the condition is called cutis hyperelastica. There is little change in the histologic appearance of the elastic network. The fibrils exhibit greater tortuosity, angulation and spiral winding than normal, but the principal abnormality seems to be in the form of split collagenous fibrils (Unna, 1896).

In treating of the influence of the endocrine secretions, one must consider the normal and abnormal imbalances of glandular activity. The remarkable changes in the elastic tissue of the uterus and its arteries during pregnancy have been described in the preceding pages. It does not seem reasonable to believe that chance abetted by mechanical forces could bring about such cyclic changes as those which occur in the elastica of ovarian vessels in the course of menstruation and ovulation. Furthermore, it is generally accepted that the development of striae gravidarum cannot be explained by any theory which rests solely on a mechanical or an inflammatory basis. Of greater interest is the fact that hypertrophic striae distensae occasionally appear in girls at puberty even though there is no unusually rapid gain in weight (Ebert). This may be linked in some fashion with the Cushing syndrome, pituitary basophilism (Cushing). In this syndrome, which is related in some way to basophilic cell adenoma of the hypophysis, the presence of purplish cutaneous striae in men as well as in women is one of the cardinal features. Dr. Merrill Sosman called my attention to a patient with this syndrome who has received roentgen treatment of the pituitary. She has shown a remarkable constitutional improvement, and the purplish striae have become pale and atrophic. Purplish striae also have been described in patients with carcinoma of the adrenal.

The elastica as affected by other endocrine disorders has received scant attention. Unna (1896) described hypertrophy of collagen without alteration of the cutaneous elastica in acromegaly. In myxedematous skin he found many basophilic fibrils and some evidence of multiplication of the elastica, although much of the connective tissue framework of the skin had degenerated. Foerster described degeneration of elastic and collagenous tissue in myxedema. The elastic fibrils were swollen,

and the collagen tended to disappear. Both elements were often fused into homogeneous hyaline masses. Reuter found mild degeneration of collagenous and elastic tissue. This was independent of edema and occurred when the basal metabolic rate was low. He stated that the histologic changes were similar to those which occur in solid edema of the extremities of patients with exophthalmic goiter. In diabetes the frequency of arteriosclerosis with attendant deterioration of the elastica of vessels is well known. Davis and Warren described hyalinization and calcification of cutaneous elastic fibers in diabetes. Orsos (1926), described deterioration of elastica in the lymph nodes of 2 diabetic patients.

Thus it seems that the nature of the elastica of the body may be influenced by congenital and hereditary factors and that in normal persons and in persons of certain constitutional types, as well as in those in various states of endocrine activity, the elastica may exhibit important variations of local or general distribution and of qualitative or quantitative nature.

SUMMARY

One hundred years have passed since elastica was identified as an elementary tissue. The belated segregation of this tissue is to be attributed in part to the fact that it is always accompanied by collagen in the mammalian body and is often associated with smooth muscle. The tissue is composed of continuous fibrils, bands and membranes. It is especially abundant in systems which undergo considerable fluctuations in size, particularly if the deformations are rhythmic.

The sequences in the formation of the elastica in the developing embryo indicate that, although the structure and distribution of elastic tissue may in part be predetermined, functional demands beginning in early embryonic life also play an important role. The maturation of elastic tissue in the embryo with respect to acquisition of form and characteristic staining reaction is a relatively slow process. This is also true in the evolution of the tissue from a phylogenetic point of view. It seems to have no important place in the structure of invertebrates and lower vertebrates, achieving full significance only in the higher vertebrates.

The histogenesis of the elastica is obscure, even though we accept a cellular theory of its origin and the broad postulate that it arises through a continuous change of endoplasm into ectoplasm. Presumably, the fibroblast is implicated in the formation of elastic tissue. If this is true, it is certain that the mechanism for the formation of the elastica is not influenced by stimuli in the same way as the mechanism for the formation of collagen.

The tissue has the physical properties by which bodies achieve high elasticity in the common sense of the term. Within the elastic limits it has great extensibility and an inherent capacity to return promptly to its original shape after the deforming force has been removed. It is not known whether any elastic fibril freed from encumbrances obeys the laws of elasticity as applied to rods and wires.

There is no proof that all structures which are classified as parts of the elastica are chemically identical. However, those elastic tissues which have been studied seem to have similar chemical properties. They are highly resistant to the action of ordinary laboratory reagents and belong to the heterogeneous class of scleroproteins. Mature elastica has high reducing power and is more acid than collagen, with which it is often associated. These properties serve as a basis for differential staining reactions.

The characteristic staining reactions are subject to wide variations. They are acquired slowly in the embryologic, phylogenetic and in vitro formation of the tissue. A change in the important physical properties or in the chemical structure of the tissue is not necessarily accompanied by a change in the morphologic aspects or in the staining reactions of the tissue. Conversely, an alteration in the staining reactions does not always signify an important change in the functional capacity or in the structural integrity of the tissue.

The tissue is of importance from the physiologic point of view. It is designed for the mechanical coordination of movement of separate units of structures with which it is associated. It aids in the dissemination of stresses which are directed at isolated points. It conserves muscular energy through partial maintenance of tone during the phase of relaxation of muscular elements. It serves as a restraining bulwark against the possible injury of excessive forces. It aids materially in the return of an organ or tissue to its natural shape after the deforming force ceases to act. Its function is always influenced not only by its inherent elastic qualities but also by the elasticity of the tissues with which it is intimately bound.

The morphologic changes which the tissue undergoes in various pathologic states have been studied thoroughly. No attempts have been made to correlate accurately the loss in the functional capacity of the tissue with changes either in morphologic appearance or in staining reactions. However, it is generally recognized that the accepted evidences of degeneration do not always accompany diminution in the functional capacity of the tissue. Degenerative changes may go to completion or be interrupted at various stages in the usual sequence of atrophy and disintegration. If degeneration has progressed to a measurable degree from a morphologic standpoint, there is limited capacity of the

tissue to regain its structural integrity. Regeneration of the tissue does occur occasionally, and fibrils may form even in regions where none was present normally. However, once the elastica in a region has disintegrated under pathologic circumstances, the replacement is usually meager and imperfect. If the normal architecture of the tissue has not been greatly disturbed in the presence of degeneration of the elastica and no inflammatory reaction has accompanied the deterioration, a fairly perfect regeneration of the elastica sometimes occurs.

Inflammatory lesions are notably injurious to the tissue, irrespective of the cause of the inflammatory reaction. Suppurative processes of acute or chronic nature, especially if they are accompanied by an excessive production of granulation tissue, are particularly deleterious. Necrosis of tissue in the absence of suppuration often permits an unusually perfect retention of the morphologic character of the tissue.

There are many conditions in which the elasticity of the fibers seems to be reduced in the absence of any significant morphologic change. These are much too numerous to mention here, but it is to be emphasized that disturbances in the functional capacity may be temporary. The function and morphologic character of the elastica have not been adequately studied in the various disorders due to vitamin deficiencies. In vitamin C deficiency there is some evidence of alteration in the staining reaction of the tissue. There are no data which are concerned with the functional capacity of the tissues in any of the deficiency diseases.

Neoplasms, especially if they are malignant, are usually free from elastic tissue even though there may be an abundant collagenous stroma. The only carcinoma which is accompanied by a significant amount of newly formed elastica is the argentaffinoma. No sarcoma has been described in which there was any evidence that the neoplastic cells had been instrumental in the production of elastic tissue. Benign cutaneous tumors have been described in which there was a large amount of poorly formed and degenerating elastic tissue. The malady in which these tumors were observed to occur is known as pseudoxanthoma elasticum; it is often characterized by angioid streaks in the retina.

There is some evidence that heredity may exert some influence on the quality and content of the bodily elastica. Elasticity of tissues varies among different persons. Hypoextensible and hyperextensible arteries occur in apparently normal subjects. Minor differences among comparable persons in the elasticity of the skin, dating apparently from early life, are common observations. An extraordinary elasticity of the skin is occasionally present in a disease which is known as cutis hyperelastica. Evidence of defective formation of elastica in the skin is the most important pathologic feature in a hereditary disease which is known as epidermolysis bullosa hereditaria.

There is good evidence that various states of activity of some endocrine glands are accompanied by alterations in the elastica. In diabetes there is commonly deterioration of elastic tissue. Perhaps the most outstanding example of the possible influence of endocrine secretions is the alteration in the elastica of ovarian vessels in the menstrual cycle. The increase of elastica in the uterine wall in the early stages of pregnancy and its rapid disappearance in the latter weeks of gestation are not explicable by the usual factors which bring about deterioration of the tissue. This is perhaps less true of the involutional changes in the walls of uterine arteries after the termination of pregnancy or in the walls of placental arteries as the period of gestation nears its termination.

The most important influences on the integrity of the elastic tissue are in some way related to age. The tissue increases in amount in the various organs over a period of several years. Then, beginning in the second decade of life, there is gradual diminution in the elasticity of the tissue. This loss of elasticity is a generalized involvement of the elastic networks, whether they are in the arteries, lungs, skin or elsewhere. In the age period between 10 and 60 years the arterial elasticity is reduced to approximately one half of that optimum which existed in the early years of life. This is independent of the atherosclerotic process. Accompaniments of the gradual decay are impregnation with calcium salts, loss of characteristic staining reactions, morphologic disintegration, and relaxation of the structures normally supported by the elastica. Thus, a large part of the visible effects of age—the functional effects in all instances and the morphologic in the majority of instances—seems to be attributable to this inexplicable deterioration of the elastica. It is particularly interesting that in placental arteries sequences identical to those described in the aging process as it affects the arteries of the average person follow in rapid succession, taking place in the short span of a few months.

In conclusion it is hoped that this survey will direct attention to a much neglected tissue. It is fair to state that no more than a beginning has been made in the study of this tissue. It may be permissible to point out that an analysis of the data indicates that future investigators may profitably concern themselves with a substance or substances which are elaborated from relatively simple chemical compounds and deposited in elective sites. It may be suggested that some of the substances from which the tissue is constructed are capable of maintaining a metabolic interrelationship with the components of the medium in which they persist. It may be predicted that these substances are of such labile nature as to react reversibly in situ and to be amenable to resorption, redeposition and augmentation in response to influences of either a local or a general nature.

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Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—Victor H. Bassett, health officer of Savannah, Ga., and at one time professor of pathology in Milwaukee, died Nov. 3, 1938, aged 67 years.

L. Corsan Reid has been promoted to the rank of assistant professor of pathology in the New York Medical College.

Thomas Francis Jr., member of the staff of the International Health Division of the Rockefeller Foundation, has been appointed professor of bacteriology in the New York University College of Medicine.

Society News.—The annual meeting of the American Association of Pathologists and Bacteriologists will be held at the Medical College of Virginia, Richmond, Thursday and Friday, April 6 and 7, 1939. The headquarters will be Hotel Jefferson. The secretary is H. T. Karsner, 2085 Adelbert Road, Cleveland.

The sixty-eighth annual meeting of the American Public Health Association will be held in Pittsburgh, Oct. 17 to 20, 1939, with headquarters at the William Penn Hotel.

The fiftieth annual meeting of the Association of American Medical Colleges will be held at Cincinnati, Oct. 23, 24 and 25, 1939.

The American Board of Pathology announces that it has changed the date of its next examination from April 8 to 9 to April 3 to 4.

The Scandinavian-American Foundation is interested in an exchange of medical students between Scandinavian countries and the United States. The Association of American Medical Colleges will cooperate.

CORRECTION

In the article by Dr. Harold Wood entitled "A Tray for Dehydration, Clearing and Paraffin Impregnation of Large Quantities of Tissue," in the August issue (ARCH. PATH. **26**:532, 1938), "75" should be substituted for "225" in the fourth line of the first paragraph under the center head "Description of Tray."

Obituaries

HERBERT U. WILLIAMS, M.D.

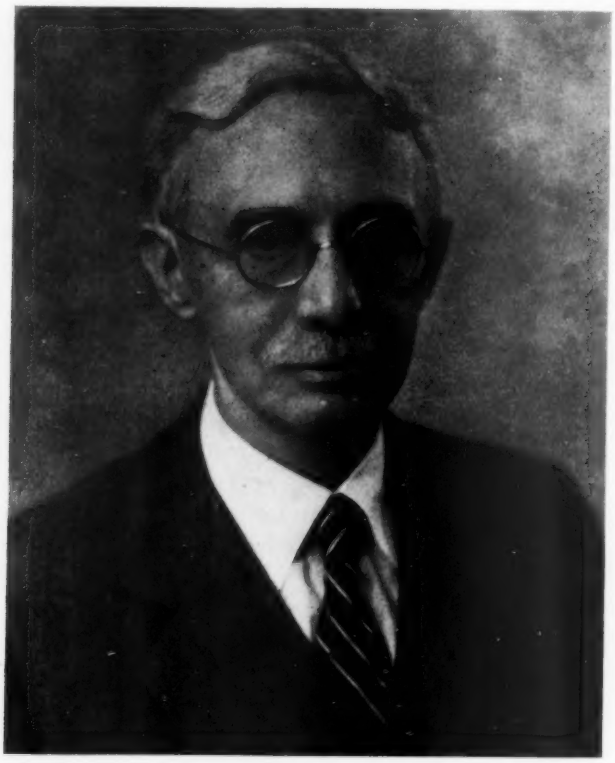
1866–1938

Dr. Herbert U. Williams retired from teaching four years ago. He used to spend the winter months in warmer climates, in central and southern states, in the leisurely pursuit of his pathologic studies of ancient diseases. Again he was planning to go south, for he enjoyed excellent health until very recently. He was hardly aware of harboring an organic lesion when, late in the evening of December 8, last, while greeting friends at his favorite club, he was suddenly seized with a heart attack. Shortly after, still completely unconscious, he died at the Buffalo General Hospital, the hospital in which his career had started. Postmortem examination revealed marked sclerotic aortic stenosis, with distinct narrowing of the coronary ostia, recent pulmonary edema and marked dilatation of the stomach, with constriction from chronic ulcer at the pyloric ring.

Williams, who was a native of Buffalo, was born Nov. 28, 1866. He graduated from the old Central High School, going from there to the University of Michigan for two years. In 1886 he entered the medical school of the University of Buffalo and obtained his medical degree in 1889. He continued his medical studies at the University of Pennsylvania, where he obtained a doctorate in 1891. Postgraduate studies then took him to the College of Physicians and Surgeons in New York, to the Johns Hopkins Hospital (in the department of William Welch) and to the Pathologic Institute of Johannes Orth in Göttingen, Germany. In 1894 Williams was appointed professor of pathology and bacteriology at the University of Buffalo; soon after, he became head of the department. In this capacity he served the medical school for forty years, until his retirement in 1934. From 1912 to 1915 he was dean of the medical school.

The early scientific papers of Williams concerned the nature of fat necrosis—its morbid anatomy, causation and experimental production. In the field of experimental pathology Williams was one of the early pioneers. By ligating the pancreas in cats, he produced marked fat necrosis. However, the positive results were largely enhanced by infection, and further experiments were carried out to eliminate the role of infection. Pancreatic tissue was inserted in the subcutis of cats by means of a special glass cannula. He was then able to produce fat necrosis in a number of instances in which infection was eliminated.

The histologic changes were carefully analyzed and described. These and other experiments verified the conclusion of Hildebrand that the mere action of the ferments of the pancreas brings about fat necrosis. In his control studies Williams found that fat necrosis occurred also spontaneously in different animals. Other experiments dealt with the causes and genesis of acute pancreatitis. In these he placed in the bile ducts glass cones, which he pushed through the common duct in imita-



HERBERT U. WILLIAMS, M.D.
1866–1938

tion of the passage of gallstones. Then he injected duodenal content into the pancreatic duct through a blunt cannula. By these methods he produced acute necrosis of the pancreas in a few cases and concluded that regurgitation of the duodenal content in the diverticulum of Vater, the way having been opened by the passage of stones, leads to the entrance of enterokinase into the pancreas, resulting in digestion of pancreatic tissue and necrosis. He was unable to produce these effects with an injection of sterile enterokinase alone. In another paper he

stressed the role of traumatism in necrosis of the pancreas following operation on the gallbladder.

Williams also studied the new formation of elastic fibers in the stroma of carcinoma at the time when the Weigert stain had just become known. In his paper on this subject, as well as in a critical review of the literature on plasma cells and mast cells, it is evident that he had a considerable command of technical methods. Much of his description of the granules in mast cells, observed after the use of different fixatives, is based on his own experience. Other, mostly morphologic studies concern the histologic changes in the sweat glands in chronic nephritis, the incidence of trichinosis in routine postmortem material and the lymphomatous tumors or tumor-like nodes in the normal dog's spleen. In the latter instance he made a systematic investigation of the spleens of 720 dogs. A few brief papers of bacteriologic interest might be mentioned: one concerned a mixed Welch bacillus–Streptococcus infection causing pyelitis; a second, results of attempts to cultivate trypanosomes from frogs; a third, sputum examination during the influenza epidemic, and a fourth, experimental studies of the agglutination of human erythrocytes by horse serum. He also wrote a manual of bacteriology, mainly for the use of medical students, which had five editions.

In the years following the great war Williams became increasingly interested in the history and antiquity of diseases, especially in the history of syphilis. To the readers of the ARCHIVES OF PATHOLOGY his reviews on paleopathologic topics and on the origin and antiquity of syphilis are well known. These studies brought Williams a world-wide reputation among pathologists, medical historians and anthropologists alike. In the field of human paleopathology he published original observations on a peculiar symmetric osteoporosis which he had found chiefly in ancient skulls from Indian children. These he found especially in the areas where the cultivation of Indian corn was most highly developed. He felt that this osteoporosis was possibly allied to rickets or scurvy or some types of anemia in children, as the effect of a lack of vitamins or of other dietary deficiency. It had not been found in skulls of white people. This review included diseases known in modern times—for instance, tuberculous bone lesions in a neolithic skeleton (from Europe) and in a mummy of a priest of Amen. He examined two Peruvian mummies and found distinct evidence of arteriosclerosis with a calcified thrombus. Deforming arthritis, he found, was apparently common among ancient people, as well as biliary and urinary calculi, cirrhosis of the liver and small osteomas; however, according to Williams, dental caries was less common in ancient times and became more frequent with the alteration of living conditions in modern civilization.

In his review of the origin and antiquity of syphilis Williams not only analyzed critically all reported cases of ancient syphilis of the bone, many of which he had studied personally in the large museums of Europe and America, but he added entirely new material from the American continent. He gave an excellent description of syphilitic lesions of the skull and long bones and of the difference between these and the lesions of Paget's disease. In his opinion, careful examination with the unaided eye, especially of the long bones sawed lengthwise, gave more information than any other method. Many ancient bones showing periosteal new growth were examined histologically.

As Williams had traveled a great deal and had visited many museums of pathology both here and abroad, he had a large material at hand for a comparison of pre-Columbian skulls, such as that in the famous Pecos case, with skulls from cases proved to be instances of syphilis, at the museum in Prague. Study of a very large material of ancient skulls and long bones, which was collected in different parts of the Americas, especially New Mexico, Peru, Argentina, Florida, Ohio, Tennessee and Louisiana, led Williams to conclude that the Indians were afflicted with syphilis before the arrival of the white man. It was his belief that no similar proof from the Eurasian continent had been produced; no convincing case of syphilis in Europe can be dated previous to 1500. In further proof of the American origin of syphilis Williams made citations from early Spanish authors concerning "bubas" as collected by Montejo Y Robledo. From all these presentations it seems evident that syphilis was not known in Europe prior to the discovery of America or to a few years preceding that time, when the Portuguese were exploring the coast of Africa. It was apparently the question how far bone lesions in yaws might simulate those of syphilis, and its bearing on the analysis of ancient bones, which induced Williams to study yaws in various tropical islands—Haiti, Java, the Philippines. He found, however, very few evidences of bone diseases in yaws. In his review of yaws and syphilis he gave a complete histologic analysis of the primary and secondary stages in yaws and syphilis, stressing in the former especially the marked proliferation of the epithelium and the large number of leukocytes penetrating the epithelium, along with but slight involvement of blood vessels. He did not find any evidence that internal viscera were infected in yaws.

Williams was an excellent teacher, beloved and esteemed by his students through the forty years of his teaching. He tried to coordinate the teaching of pathology with that of the major medical disciplines. The University of Buffalo owes him a large debt of gratitude, not only for this, but because it was largely due to his efforts that the school of arts and sciences was successfully inaugurated in 1913, when Williams was dean of the medical school.

Williams was a member and former president of the American Association of Pathologists and Bacteriologists and a member of the American Association of Physical Anthropologists and of the American Anthropological Society. He was a fellow of the American Association for the Advancement of Science.

It was in the dark corridors of the German Pathological Institute in Prague that I first met Dr. Herbert U. Williams, about twelve years ago. He was on one of his scientific excursions, visiting the museums of the pathologic institutes in Paris, Berlin, Vienna and Prague. His kind manner and most vital interest in his scientific objectives, his seemingly timeless enthusiasm, made on all of the younger men an unforgettable impression. And so he will be remembered by countless students, the colleagues whom he helped and his fellow pathologists, here and abroad, for his teaching, his remarkable personality and straightforward character, and for his scientific work.

KORNEL TERPLAN.

GEORGE BURGESS MAGRATH, M.D.

1870—1938

George Burgess Magrath, born Oct. 2, 1870, was the only son of Rev. John Thomas and Sarah Jane (née Herrick) Magrath, natives of Maine. The former was graduated from Bowdoin College.

Born in Jackson, Mich., he moved with his parents in 1877 to Battle Creek, in 1879 to a suburb of Philadelphia, in 1882 to Hyde Park, Mass., and in 1890 to Milton, Mass., with the changing of pastorates.

In Hyde Park he went to high school three years and studied the organ under John A. Preston. Later he became the organist at the Church of the Holy Spirit, which was his father's charge.

While living in Milton, he went to the Roxbury Latin School and graduated at the head of his class, although he entered six weeks late.

He entered Harvard University in 1890. He graduated in 1894 magna cum laude, with the degree of Bachelor of Arts, and was speaker of his class. While here he became identified with athletics, especially with rowing and sculling. He joined the Union Boat Club in 1898 and from 1899 to 1917 he rowed every year on club crews. In 1907 he was on the winning senior double scull shell crew of the club in the July Fourth Regatta, and it was a familiar sight to see him pulling an oar every day in a shell on the Charles River.

In 1898 he graduated from Harvard Medical School cum laude, but before he graduated (January 1898) he worked at the Boston City Hospital in the pathologic department, under Councilman's direction, until October 1898. Then he became one of Councilman's assistants in the pathologic department of the Harvard Medical School, and there under a Bullard Fellowship he completed a research study which gained him the degree of Master of Arts in 1899. After that came concurrent appointments at the Carney, Long Island, St. Elizabeth's, Cambridge and Faulkner hospitals, where he was able to "give some help in developing the pathological laboratory as a necessary adjunct to the modern hospital."

During this time an epidemic of smallpox occurred, and in the work on this Dr. Magrath "participated," completing his work in 1903 and announcing the discovery of Cytorrhyctes variolae in his joint study on the pathologic nature and etiologic factors of variola and of vaccinia with W. R. Brinkerhoff and I. R. Bancroft. A collection of 28 care-

fully prepared specimens of skin from persons involved in the epidemic are to be found in the Warren Anatomical Museum of Harvard Medical School and attest the active postmortem study of the disease at that period. One recognition of this work was an offer of a professorship in pathology at Washington University, St. Louis, but this he

George Burgess Magrath

1870—1938

declined. Continuing in the department of pathology and becoming assistant to the secretary of the Massachusetts State Board of Health, he investigated outbreaks of communicable diseases.

In 1905 he changed from being assistant in pathology to that of assistant in hygiene at the Harvard Medical School and in 1907 was

selected to follow Dr. Francis A. Harris as medical examiner for Suffolk County by the then Governor Curtis Guild. Dr. Magrath expressed himself in regard to this office at the time as follows:

> The duties of this office consist chiefly in the investigation of deaths due to injury of any sort and of those which are sudden or unexplained; they necessarily include service from time to time in court. The position is one affording wide opportunity for use in the service of the state of the knowledge and technical skill yielded by special training and experience. In doing my work I have sought to apply to the branch of state medicine, which my office represents, the result of the generous type of scientific medical education which it was my good fortune to receive. Since 1907, I have been appointed instructor in legal medicine. Teaching in this subject is by lecture and by demonstrations to members of the third and of the forth year classes of the medical school. The general standard of medical jurisprudence in this country is none too high and it is my aim to help raise its level by applying to my own work the principles and the methods of modern scientific medicine and by impressing on the student the importance of the responsibility of the physician in all matters wherein medicine is brought into the service of the law.

Dr. Magrath was able to interpret General Laws, chapter 38, Medical Examiners, which was written in a vague and general manner, for the best interests of the situations he was called on to meet.

He labored with all forces to establish rules of practice which would extend the provisions of this law. These rules have been variously adopted and have become customary.

In 1908 and in 1910 he made short trips to England, France and South America. In 1914 and 1915 he assisted in the framing of legislation and in creating for the City of Greater New York a system of medicolegal inquiry based on the plan of that which he developed in Suffolk County, Mass., and he was invited to be a candidate for the position of Medical Examiner but refused.

He said: "Outside of official and academic duty I am from time to time engaged as medico-legal pathologist or consultant in matters medico-legal and in this capacity I have participated in numerous murder trials, some of them in other states. In such instances I serve only at the call of the government."

It is to be remembered that with all this fatiguing industry he was as active as ever in directing societies and clubs, in rowing and in music, his diversions.

In the World War, while desiring to go, he felt his service was urgently needed at his own "post of duty," but he did accept in 1918 the commission of Major in the Medical Corps, Massachusetts State Guard. He was "prepared to respond with my assistants and ambulance service, to call from Acting Chief Surgeon, Massachusetts State Guard, in the event of any disaster involving loss of life."

From 1919 on, his activities increased in the matter of participation in investigations of mysterious deaths. How extensive these were will be remembered by those who attended his lectures to the students or his Lowell Lectures in 1932, or heard addresses given by him before the Massachusetts Medico-Legal Society. A postmortem examination skilfully and painstakingly done with double measurements, metric and linear, was only one part of such duty. *"One must investigate the premises, know all the circumstances, use judgment and common sense, and in the pursuit of the truth be relentless."*

In 1931 a chair of medicolegal medicine was endowed at the Harvard Medical School and he was made professor at that time, having previously been instructor in the subject since 1907.

In 1935 he resigned from the office of Medical Examiner and in 1937 was made emeritus professor of medicolegal medicine at the Harvard Medical School.

An unfortunate infection of both his hands crippled his action and reduced his activities, but he filled his new leisure with precautions for his health and with enjoyment of his varied interests, particularly that of music. Indeed, he was preparing to attend a concert when he fell ill, on Dec. 11, 1938, with the cerebellar hemorrhage from which he died.

MYRTELLE M. CANAVAN.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Pathologic Changes Produced by Gastrectomy in Young Swine. S. Petri, F. Nørgaard and J. Bing, Am. J. M. Sc. **195**:717, 1938.

In 6 week old pigs operative removal of the stomach gave rise to a number of severe clinical and pathologic anatomic changes that may be interpreted as similar to those found in human pellagra. Conspicuous features in this condition were arrest of growth, hypochromic microcytic anemia and extensive changes in the skin and in the central nervous system. In addition, there were diverse inconstant changes, as hyperproteinemia, plasma cell metaplasia of the spleen and lymph glands, cirrhosis of the liver and osteoporosis. In principle, the authors state, these results correspond to their observations in studies on pups, suggesting the presence of a specific factor in the stomach of swine, dogs and man which is required to maintain the skin and central nervous system in a normal healthy state.

From Authors' Summary.

Experimental Rickets. G. S. Dodds and H. C. Cameron, Am. J. Path. **14**:273, 1938.

Rickets was produced by the Steenbock-Block diet; healing was brought about mainly by administration of viosterol. Healing is described in detail from study of sections stained with hematoxylin and eosin, silver preparations and roentgenograms which were made frequently. The twelve roentgenologic stages of Bourdillon were used as a scale for measuring the progress of healing. The first indication of healing was calcification in the rachitic metaphysis, close to the edge of the epiphysial cartilage, whence it spread, first through the metaphysis toward the shaft and later into the cartilage toward the end of the bone. The preliminary reorganization of the metaphysis involved: provisional dilute calcification of the osteoid; calcification of the exposed cartilage trabeculae; increase in the amount of marrow, and restoration of the marrow structure to normal. These changes restored the configuration of the trabeculae to what was almost normal by exposing the rather parallel cartilage trabeculae. The end of the shaft underwent much the same changes. At the same time the projecting cartilage masses became calcified and were partly removed, leaving calcified trabeculae very similar to those in the metaphysis. During the latter half of healing the shape of the bone became corrected; hematogenic marrow formed in the new end of the shaft, which had been formed from the metaphysial region; cupping was corrected, the entire epiphysial cartilage became normal in thickness and structure, and the reorganized trabeculae attained normal dense calcification. From Authors' Summary.

Experimental Bronchiectasis. W. E. Adams and L. Escudero, Tubercle **19**:351, 1938.

Bronchiectasis was produced experimentally in dogs. Two factors were necessary for its production, namely, incomplete bronchial obstruction and infection of the bronchi. It was produced by both aerobic and anaerobic pyogenic organisms, by tubercle bacilli and by the organisms causing distemper. Incomplete obstruction was essential; at no time did bronchiectasis follow complete bronchial obstruction. An increase in intrapleural negative pressure was important only so far as the

degree of severity of the pathologic changes was concerned; when it was present, bronchiectasis developed to a somewhat marked degree. Thus, in the explanation of this condition, the original theory of Laënnec appears to be the one most nearly correct.

H. J. CORPER.

THE GROWTH OF HUMAN BONE MARROW IN TISSUE CULTURE. G. WEITZMANN and EVA POSERN, Virchows Arch. f. path. Anat. **299**:458, 1937.

The technic of the culture of human hemopoietic tissues was described by Meier, Posern and Weitzmann in the preceding issue of *Virchows Archiv* (previously abstracted). The present authors proceed to the description of the growth and differentiation of a single cell type in their cultures. It has been found possible to grow marrow removed by sternal puncture, thus greatly broadening the scope of the study of marrow. The marrow of patients with chronic or cachectic disease grows more slowly than the marrow of normal persons or that of persons with acute disease. In the growth and differentiation of the cell type selected for study, four stages are described: In the first stage, that of emigration of cells from the explant into the peripheral growing zone, there are seen round, oval or polygonal cells that differ from blood cells in the finely granular and dark character of their cytoplasm. The granules give a lipoid-staining reaction with nile blue sulfate; they are not the result of degeneration but represent lipoids formed in the intermediary metabolism of the cells. In the second stage the granulation of the cytoplasm disappears, the cells grow into giant cells, twenty to fifty times the size of the myeloid cells among which they lie, and the latter are filled with phagocytosed cells. In the third stage the cells become smaller, elongated and mononucleated, and phagocytosis is less active. From the poles of the cells processes grow out, which unite with those of other cells to form a wide-meshed network. The fourth stage is characterized by formation of fibrils. The cells whose life history has been thus followed in tissue culture are derived from the reticulum cells of the bone marrow.

O. T. SCHULTZ.

EXPERIMENTAL ALLERGIC HYPERERGIC ARTERITIS. W. RINTELEN, Virchows Arch. f. path. Anat. **299**:629, 1937.

Rabbits were sensitized by repeated subcutaneous injections of horse or swine serum. In the sensitized animals and in controls the carotid artery was exposed and doubly ligated under ethyl carbamate (urethane) and ether anesthesia, and the provocative dose of the antigen was injected into the lumen of the artery between the two ligatures. Within four hours fibrinoid swelling of the wall of the vessel was evident in the sensitized animals. This was followed by leukocytic infiltration. By the second day there was a marked histiocytic reaction. The inflammatory process involved the entire wall. The process usually ended in thrombosis of the artery, with replacement of smooth muscle and elastic tissue by fibrous tissue. In control animals the injection of serum was followed by only a slight perivascular inflammatory reaction, resulting from the trauma. Normal horse serum from a commercial concern did not produce hyperergic arteritis such as that described.

O. T. SCHULTZ.

Pathologic Anatomy

EXPERIMENTAL PNEUMOCOCCUS INFECTION IN TUBERCULOUS RABBITS. J. WEISSFEILER, E. N. MOROZOVA and A. I. STRUKOV, Am. Rev. Tuberc. **37**:93, 1938.

Rabbits infected intratracheally with virulent strains of tubercle bacilli of bovine type cope with an intravenous or an intraperitoneal inoculation of pneumococci of type I as well as control animals. The cellular reaction in a tuberculous animal clearly differs from that in a healthy rabbit. The reaction of the tuberculous animal takes a slower course, has a more exudative character and tends

more toward necrosis than the reaction of the healthy rabbit. The difference observed in the reactions of tuberculous and healthy animals can be explained by heteroallergy due to tuberculous infection. Thus, heteroallergy plays an important part in the course of nonspecific infectious processes in a tuberculous organism.

H. J. Corper.

Bundle Branch Block. W. M. Yater, Arch. Int. Med. **62**:1, 1938.

Bundle branch block is usually due either to disease of the coronary arteries (rheumatic or degenerative) or to hypertension resulting in strain of the left ventricle and impairment of the nutrition of the endocardium and bundle branch. Bundle branch block is as a rule associated with lesions of both bundle branches although one branch is usually more seriously affected than the other and probably determines the essential form of the electrocardiographic curve. The newer, or American, terminology is more nearly correct for bundle branch block in man, although it must be admitted that whether "right" or "left" is used to modify the term designating the diagnosis of this disturbance in conduction, the adjective merely indicates the branch more seriously affected. The uncommon form of bundle branch block, right bundle branch block, is probably due as a rule to rheumatic arteritis or rheumatic myocarditis. The common form of bundle branch block, left bundle branch block, is probably due as a rule to degenerative cardiovascular-renal disease, meaning coronary arteriosclerosis or arterial hypertension or both. A bundle branch need not be entirely destroyed at any level in order to produce electrocardiographic alterations that may be designated as typifying bundle branch block. An increased amplitude of the ventricular complex is not essential to the electrocardiographic diagnosis of bundle branch block. Any increase of the QRS interval beyond one-tenth second may indicate lesions of the bundle branches. Many questions remain unanswered in regard to bundle branch block, and many careful histologic studies must be made before most of them can be answered.

From Author's Summary.

Periarteritis Nodosa. J. W. Kernohan and H. W. Woltman, Arch. Neurol. & Psychiat. **39**:655, 1938.

Pathologic studies of 5 cases of periarteritis nodosa convinced Kernohan and Woltman that the primary lesion is in the media of the smallest arteries (periarterioles). The media first undergoes hyaline-like degeneration with necrosis, followed by changes in the adventitia and intima. The adventitia becomes infiltrated with lymphocytes, plasma cells and endothelial-like elements; the intima proliferates, causing narrowing and even obliteration of the vascular lumen. It may become separated from the media by lymphocytes, plasma cells and endothelial cells. The vascular lesions were usually present in every organ, but in a single case they were limited to the peripheral nerves; in another case, they were also very marked in the brain, and in a third case they were pronounced in the retinal blood vessels (the arterioles of the choroid). The changes in the peripheral nerves showed as swelling degeneration and complete disappearance of the myelin, followed by degeneration of the axons. In addition, there were infarcts in the nerve trunks, multiple and single. Kernohan and Woltman consider the changes in the nerves secondary to those in the blood vessels, the result of an inadequate supply of blood.

George B. Hassin.

Regional Ileitis and Ulcerative Colitis. E. S. Stafford, Bull. Johns Hopkins Hosp. **62**:399, 1938.

Stafford discusses 10 cases in which regional ileitis, and 3 in which ulcerative colitis, was associated with lymphogranuloma. The similarity of the clinical features in these two types of cases has been noted. The typical microscopic lesion of lymphogranuloma is differentiated from the nonspecific lesions of regional

ileitis by the presence of the tubercle-like group of epithelioid cells with a central core of polymorphonuclear leukocytes. That the virus of lymphogranuloma may cause the ulcerative lesions in the colon has been considered, and the possibility suggested that the lesions of regional ileitis may also be due to the virus.

FROM AUTHOR'S SUMMARY.

VARIATION IN RETICULOCYTES OF RABBITS. P. NICOLLE, Arch. Inst. Pasteur de Tunis **27**:42, 1938.

In rabbits inoculated with classic typhus the reticulocyte count increases from 2 or 3 per hundred red cells to 6 or 7 within from ten to fourteen days.

Rabbits inoculated with Trypanosoma congolense present an anemia accompanied by an increase in reticulocytes. There is a rapid decrease in the red blood cell count for from eleven to nineteen days, followed by a chronic stage and eventually by a return to normal. As the red blood cells fall below 3,000,000 per cubic millimeter, the reticulocytes increase to 450,000 per cubic millimeter. There is marked leukopenia with a proportional increase in mononuclear cells. Polychromatophilia, poikilocytosis and anisocytosis are common. The anemia is one of cell destruction without permanent lesions of the hemopoietic organs.

During the period of incubation of experimental infection with Trypanosoma equiperdum there are successive waves of reduction of the red cell count and increase in the number of reticulocytes, with periods of return to normal. When the number of red blood cells decreases from 20 to 25 per cent that of reticulocytes increases from 50 to 100 per cent.

Spontaneous parasitic infections and trauma may also alter the unstable reticulocyte equilibrium of the rabbit.

J. B. GUNNISON.

INTRANUCLEAR FORMATION OF MELANOTIC PIGMENT. K. APITZ, Virchows Arch. f. path. Anat. **300**:89, 1937.

In a study of melanosarcoma and pigmented moles Apitz describes the formation of melanotic granules within the cell nucleus. His concept of pigment formation is that the nucleus furnishes to the cytoplasm an invisible fluid material (melanogen) which is transformed into visible pigment in the cytoplasm. In neoplasms the melanogen may be retained within the nucleus and may there be transformed into pigment. Such formation of pigment within the nucleus occurs always within vacuoles. The latter he considers characteristic of pigment-forming cells and believes that they may help determine the melanotic origin of a nonpigmented metastasis.

O. T. SCHULTZ.

MEDIASTINAL CYST LINED BY GASTRIC MUCOSA WITH PERFORATING PEPTIC ULCER. C. BOESS, Virchows Arch. f. path. Anat. **300**:166, 1937.

To 4 previously reported cases of mediastinal cyst lined by gastric mucosa the author adds a fifth. This cyst occurred in a boy 3¼ years old. It was the size of a hen's egg. A chronic peptic ulcer of the lining, which evidenced secretory activity of the mucosa, perforated into the left lung and led to death from hemorrhage. The most likely explanation of the malformation is that it originated from misplaced gastric mucosa or from an endodermal anlage of the esophagus with heterotopic potency. Also, origin from a remnant of the omphalomesenteric duct is held to be possible.

O. T. SCHULTZ.

PRIMARY THROMBOSIS OF THE DEEP VEINS OF THE LEG. R. RÖSSLE, Virchows Arch. f. path. Anat. **300**:180, 1937.

Primary autochthonous venous thrombosis is stated by textbooks to occur most frequently in the pelvic and femoral veins. Clinical evidence indicates that thrombosis of the plantar veins and of the deep veins of the leg is frequent.

While still at Basel, Switzerland, Rössle systematically investigated the deep veins of the calf of the leg in 400 consecutive necropsies in a period of five months. Of the subjects, 324 were over 20 years old; 88 of these revealed thrombosis of the deep veins of the leg. Thrombosis was frequently associated with degenerative and atrophic changes in the muscles of the calf. Rössle mentions three possibilities to explain this association: (1) that the changes in the muscles were due to the thromboses, against which is the fact that the thromboses were usually more recent than the muscle changes; (2) that degenerative changes in the muscles led to the formation of substances that caused the local thromboses, and (3) that the thromboses and muscle changes were independent of each other but resulted from a common cause.

O. T. SCHULTZ.

CHANGES IN THE CALF MUSCLES FOLLOWING VENOUS THROMBOSIS AND PROLONGED CONFINEMENT IN BED. H. VOEGT, Virchows Arch. f. path. Anat. **300**:190, 1937.

Rössle's observation of the frequent association of thrombosis of the deep veins of the leg with degenerative changes in the muscles of the calf led to further investigation especially from the standpoint of the effects of long confinement in bed. The muscles of the calf were examined histologically and compared with the rectus abdominus and biceps muscles. In 5 persons with venous thrombosis the calf muscles showed degenerative changes and atrophy. However, in 24 persons without thrombosis who had suffered long confinement to bed equally severe changes were observed in the calf muscles while such changes were not to be seen in the biceps and rectus muscles. Pressure, circulatory changes and inactivity are held responsible for the changes described. That the degenerating muscle tissue may liberate thrombogenic substances that lead to terminal thrombosis is admitted as a possibility.

O. T. SCHULTZ.

MASS AND SURFACE DEVELOPMENT OF THE FETAL BRAIN. H. H. MEYER, Virchows Arch. f. path. Anat. **300**:202, 1937.

The brains of 180 fetuses were studied between the fifth and tenth month of pregnancy as to changes in weight in reference to fetal length and as to the time of appearance and development of the more important sulci and convolutions. The time of appearance of the main sulci and the peculiarities of their development are summarized in a series of tables, the material being grouped according to weeks of duration of pregnancy as determined by fetal length. In their earliest development the sulci are bilaterally symmetric, but the symmetry is disturbed by the formation of anastomoses and other developmental changes. There is no direct correlation between the general development of the body and the development of the brain. The sulci of the brains of female fetuses revealed a greater degree of variation as the result of anastomosis between sulci than did brains of male fetuses.

O. T. SCHULTZ.

Pathologic Chemistry and Physics

COMPOSITION OF HUMAN BONE IN CHRONIC FLUORIDE POISONING. W. A. WOLFF and E. G. KERR, Am. J. M. Sc. **195**:493, 1938.

In a case of chronic fluoride poisoning the bones contained normal amounts of calcium, phosphorus and carbon dioxide. The fluorine content was increased up to twenty times the normal and was unequally distributed, being highest in the vertebrae and lowest in the long bones. The specific gravity of the femur was low. Data on man and animals are compared.

FROM AUTHORS' SUMMARY.

THE CEVITAMIC ACID CONTENT OF FETAL BLOOD. P. MANAHAN and N. J. EASTMAN, Bull. Johns Hopkins Hosp. **62**:478, 1938.

The concentration of ascorbic acid in fetal blood is regularly almost three times that in the maternal blood. Raising the concentration of ascorbic acid in the maternal blood by antepartum administration of orange juice raises concomitantly the concentration in the fetal blood, the ratio between the concentrations in the two bloods remaining similar. The high relative concentration of ascorbic acid in fetal blood suggests that the placenta exerts a selective action in respect to this vitamin.

FROM AUTHORS' SUMMARY.

THE INFLUENCE OF INFLAMMATION ON THE ABSORPTION OF SUBSTANCES OF VARIED DIFFUSIBILITY. R. G. MILLER, J. Exper. Med. **67**:619, 1938.

Inflammation retards the absorption of horse serum globulin and crystalline egg albumin from the peritoneal cavity and from the subcutaneous tissue, but the retardation of the absorption of crystalline egg albumin is less than that of globulin, which is less diffusible. Inflammation retards the absorption of the specific polysaccharide of Pneumococcus type I from the peritoneal cavity; inflammation may accelerate but does not hinder the absorption of dextrose from the peritoneal cavity. Inflammation retards the spread of trypan blue in the skin but accelerates absorption from the skin of a more diffusible dye, bromphenol blue. Phenol red is excreted in the urine with equal rapidity after injection into a normal and into an inflamed peritoneal cavity. Direct extractions of phenol red from inflamed subcutaneous sites indicate that inflammation accelerates the absorption of the dye from these areas. Inflammation retards the absorption of the indiffusible proteins, carbohydrates and dyes; it tends to accelerate the absorption of the diffusible carbohydrates and dyes.

FROM AUTHOR'S SUMMARY.

A COMPARISON OF THE INTERCHANGE OF THE BODY FLUIDS AFTER INTRAVENOUS INJECTIONS OF CRYSTALLOIDS, GUM ACACIA AND BLOOD SERUM. J. D. ROBERTSON, Brit. J. Exper. Path. **19**:30, 1938.

When hypertonic solutions such as 33 per cent sodium chloride or 50 per cent dextrose are injected intravenously at the rate of 5 cc. per kilogram, there is an enormous increase of the blood volume, often reaching a maximum of double the original volume. The increase takes place rapidly and only during the period of the injection. The moment the injection is stopped, the blood volume begins to fall rapidly, and in about half an hour it is normal. When 2 per cent, 0.9 per cent, 0.6 per cent and 0.3 per cent sodium chloride are injected intravenously, fluid leaves the vascular system during the injection, so that the blood volume immediately after completion of the injection is less than the theoretic level expected. After the injection the blood volume falls, but at the end of an hour it is still about 15 per cent above normal. The result of an intravenous injection of blood serum is similar to that of an injection of hypotonic, isotonic or 2 per cent sodium chloride. In the mammalian circulation sodium chloride added to acacia depresses the power of the acacia to attract fluid. When the concentration of sodium chloride exceeds 1 per cent, the attraction of fluid during the injection is greater than can be accounted for by either the acacia or the sodium chloride alone. In studying changes in the blood volume of a cat after intravenous injections of 6 per cent acacia in solutions of sodium chloride of various strengths, it was found that 6 per cent acacia in physiologic solution of sodium chloride most nearly resembles blood serum. After a hemorrhage an injection of blood serum produces in the blood volume a response similar to that after an injection of 0.9 per cent sodium chloride; and in both cases the blood volume falls rapidly after the injection. Acacia, on the other hand, produces a much slower fall in the blood volume. Acacia is retained intravascularly to a greater extent than blood serum.

FROM AUTHOR'S SUMMARY.

PANCREATIC SILICIC ACID. W. KASTEN, Frankfurt. Ztschr. f. Path. **50**:42, 1937.

In a series of 79 cases Kasten was unable to find any correlation between the silica content of the pancreas and diabetes, malignant tumor or tuberculosis.

OTTO SAPHIR.

CHEMICAL ORIGIN OF THE HISTOLOGIC SPECIFICITY OF TUBERCULOUS INFLAMMATION. F. ROULET and K. BLOCH, Virchows Arch. f. path. Anat. **298**:311, 1936.

When the acid phosphatide obtained by Anderson's method of extraction of the tubercle bacillus is purified, a magnesium salt of the compound is obtained that contains no nitrogen and a constant proportion of phosphorus. This substance when injected intracutaneously leads to the formation of a histologically characteristic tubercle or granuloma, with giant cells. This characteristic tissue reaction is related to the chemical composition of the phosphatid compound, which is taken up in finely dispersed form by the connective tissue cells; these are transformed into epithelioid cells. The phosphatide compound of the tubercle bacillus differs from the phosphatides of other plants in the composition of its saturated fatty acids, to which, when these are liberated by intracellular hydrolysis, the tissue reaction in the last analysis is due. The phosphatide compound obtained from the human bacillus causes a more marked inflammatory reaction in the human skin than in that of the rabbit, and, conversely, the rabbit's skin reacts more strongly to the phosphatide of the bovine bacillus than does that of the human being. This is interpreted as evidence of the chemical specificity of the compound in each of the two strains of bacilli. The reaction is no greater in infected (sensitized) rabbits than in normal ones, which is proof that the inflammatory reaction to specific phosphatide is not the same as the reaction to tuberculin (protein).

O. T. SCHULTZ.

VARIATIONS IN THE CONTENT OF ASCORBIC ACID AND PIGMENT IN THE HEART MUSCLE. K. UHLENBROOCK and R. BOEHMIG, Virchows Arch. f. path. Anat. **299**:699, 1937.

Previous work had shown that the lipofuscin content of the cardiac muscle increases with the functional activity of the muscle but that the increase is not uniform throughout the muscle. It had also been found that the vitamin C of the myocardium increases with the functional state of the muscle. In the present work both factors, lipofuscin content and ascorbic acid content, were determined in the same material. Because of its greater mass, the heart of the beef was selected as permitting comparison of different areas of the myocardium. The areas of the left ventricle selected were the subepicardial muscle layer, the subendocardial layer, the intervening main muscle mass and the main papillary muscle. A definite difference in content of ascorbic acid was detected, the outer muscle layer being richer in this substance than the rest of the myocardium. Variation in lipofuscin content was also noted; the areas of myocardium poorest in vitamin C were richest in lipofuscin. Vitamin C is a catalytic activator of cellular metabolism. Because of the relation of vitamin to pigment the authors conclude that lipofuscin is identical with melanin.

O. T. SCHULTZ.

EFFECT OF ASCORBIC ACID AND GLUTATHIONE ON EXPERIMENTAL DIPHTHERITIC INTOXICATION. H. LOTZE and S. THADDEA, Virchows Arch. f. path. Anat. **300**:685, 1937.

In diphtheria and other severe acute infections and intoxications the adrenal cortex may reveal evidence of damage. The adrenal cortex stores vitamin C. Exhaustion of this vitamin or of other natural reducing substances may explain the failure to obtain beneficial results from the administration of diphtheria antitoxin. In the experiments reported a large number of guinea pigs was used.

A diphtheria toxin (broth filtrate) that would certainly kill the animals in forty-eight hours or less was injected intraperitoneally. Some of the animals were given only the toxin; these served as controls. Others were given simultaneously with the toxin a wide variety of substances, chiefly protein split products. Of the many substances used, none was of any avail except the natural reducing substances of the body, ascorbic acid, cysteine and glutathione. These prolonged the life of the animals in the absence of antitoxin and prevented the changes in the liver, spleen and adrenals noted in controls. Since such changes develop very early in the course of infections and intoxications the reducing substances must be administered early if they are to have beneficial effects.

O. T. SCHULTZ.

Microbiology and Parasitology

EXPERIMENTAL INFECTION OF MICE WITH HAEMOPHILUS PERTUSSIS. W. L. BRADFORD, Am. J. Path. **14**:377, 1938.

A characteristic lesion has been produced in the lungs of mice by intratracheal inoculation of suspensions of recently isolated Haemophilus pertussis. It consists in interstitial pneumonia with appearance of an excess of mucoid exudate within the smaller bronchi and alveolar spaces. The lesion of the parenchyma is more conspicuous than that of the bronchial epithelium. Pure cultures of H. pertussis have been consistently obtained from the infected lungs of mice from ten to twenty days after inoculation. The total leukocyte count has been regularly increased, and extreme degrees of hyperleukocytosis have been observed occasionally.

FROM AUTHOR'S SUMMARY.

ABSCESS PRODUCTION BY FUSIFORM BACILLI IN RABBITS AND MICE BY THE USE OF SCILLAREN B OF MUCIN. R. TUNNICLIFF and C. HAMMOND, J. Dent. Research **16**:479, 1937.

A fusiform bacillus isolated three months previously from a patient with ulceromembranous gingivitis produced abscesses about 0.5 cm. in diameter when injected subcutaneously into rabbits and abscesses from 1 to 2 cm. in diameter when injected into skin prepared by injections of 0.1 cc. of scillaren B solution. Although cultures showed only bacillary forms, spirilla-like organisms were demonstrated in smears and sections of the abscesses. Ulceration developed in one scillaren B prepared abscess in which typical spirochetal forms were seen.

This strain of fusiform bacilli nine months after isolation did not produce abscesses in skin or peritonitis in unprepared mice; but abscesses or peritonitis resulted if the mice had previously received scillaren B intradermally or if this drug was mixed with the culture and injected intraperitoneally.

When injections of fusiform bacilli suspended in mucin were made into the skin or peritoneal cavities of mice, abscesses or peritonitis developed, but no lesions resulted from injections of mucin or bacilli alone.

More filaments and fewer spirilla-like organisms were demonstrated in lesions in mice than in lesions in rabbits, although the culture injected was often rough and contained spiral forms.

FROM AUTHORS' CONCLUSIONS.

EFFECT OF TISSUE ENZYME ON PNEUMOCOCCI. R. J. DUBOS and C. M. MACLEOD, J. Exper. Med. **67**:791, 1938.

Polymorphonuclear leukocytes contain an enzyme which destroys the basophilic character of heat-killed pneumococci (R and S variants) and inactivates the type-specific polysaccharide antigen of encapsulated cells. The same enzyme, however, fails to cause disintegration of the bacterial cells or to decompose the capsular polysaccharide itself. The enzyme has been extracted from a number of animal tissues. It appears identical with a purified enzyme extracted from pan-

creatin, and it decomposes yeast nucleic acid. These facts are considered with regard to the failure of rabbits to produce antibodies for the type-specific carbohydrate when immunized with heat-killed encapsulated pneumococci by the intradermal route.

FROM AUTHORS' SUMMARY.

CORYZA OF DOMESTIC FOWL. J. B. NELSON, J. Exper. Med. **67**:847, 1938.

Coccobacilliform bodies were regularly demonstrable in addition to Haemophilus gallinarum in exudate from birds infected with a passage strain of a coryza of rapid onset and long duration (type III). Both agents were present throughout the entire course of the disease. The characteristics of type III coryza were reproduced by injecting a mixture of the two agents. The behavior of each component was altered by the association, indicative of a synergistic relation. Evidence that the coccobacilliform bodies might occasionally develop in birds infected with H. gallinarum following transfer by indirect contact was also obtained. The combined action of the two infective agents adequately accounts for the development of this particular strain of type III coryza.

FROM AUTHOR'S SUMMARY.

CULTIVATION OF VACCINIA VIRUS WITH RETICULO-ENDOTHELIAL CELLS. J. W. BEARD and P. ROUS, J. Exper. Med. **67**:883, 1938.

The pathogenic activity of vaccinia virus is in large part suppressed when the virus is mixed with living Kupffer cells or clasmatocytes in the test tube and injected intradermally. This virus increases in quantity when introduced into such cultures in vitro and survives in immediate association with these cells. No antiviral principle is elaborated by the Kupffer cells under such conditions.

FROM AUTHORS' SUMMARY.

ORGANISMS RECOVERED FROM FILTRATES OF CULTURES OF HEMOLYTIC STREPTOCOCCI. E. E. NICHOLLS, J. Infect. Dis. **62**:300, 1938.

The results of the present study tend to confirm further the theory that when streptococci are aged under unfavorable conditions small viable forms are produced which are capable of passing through filters and which may assume visible forms when submitted to suitable conditions. Whether these filter-passing forms represent a filtrable stage of the life cycle or are merely viable particles that had occasionally escaped through the filter cannot be determined by the results obtained in this study. However, the influence of the porosity of the filter in the recovery of the organisms tends to favor the latter view.

FROM AUTHOR'S SUMMARY.

TYPE-SPECIFIC BACTERIOPHAGES FOR CORYNEBACTERIUM DIPHTHERIAE. E. V. KEOGH, R. T. SIMMONS and G. ANDERSON, J. Path. & Bact. **46**:565, 1938.

The condition produced in the skin of the rabbit by the injection of live pneumococci is inhibited by the addition of type-specific antipneumococcus serum in sufficient quantity to the dose of bacteria injected. Antipneumococcus serum inhibits the growth of the bacteria when injected with them in sufficient quantity. A method is described which permits comparison of the effects of varying doses of two antipneumococcus serums on a constant dose of live culture in the skin of the same rabbit. The potency in international units obtained by this method approaches that found by the mouse protection test. The accuracy of comparing two serums intracutaneously in one rabbit is equal to that of the mouse protection test when injections are made into 200 mice. The nature of the effect of antipneumococcus serum observed by the rabbit intracutaneous method is discussed. The two reactions described may be caused by qualitatively different properties of the serum.

FROM AUTHORS' SUMMARY.

EARLY CHANGES IN THE INTIMA OF THE INTRAHEPATIC BRANCHES OF THE PORTAL VEIN IN INOCULATION TUBERCULOSIS OF THE GUINEA PIG. F. HAUSBRANDT, Virchows Arch. f. path. Anat. **301**:223, 1938.

From five to ten weeks after subcutaneous inoculation of various strains of tubercle bacilli in guinea pigs the livers were removed, and the intima of branches of the portal vein were studied for early changes. The earliest reaction was exudative, consisting in swelling and partial desquamation of the endothelium. This was followed by swelling of the subendothelial tissues and proliferation of large subendothelial mononuclear cells. These large cells were transformed into epithelioid cells, and finally the characteristic tuberculous lesion resulted and fused with the perivascular lesion. Hausbrandt's concept of the course of events is that the bacilli penetrated the portal precapillary vessels without detectable change in the intima or media. Then a characteristic tuberculous perivascular lesion developed. The intimal changes observed developed secondary to the perivascular lesion and were not the result of localization of blood-borne tubercle bacilli directly in the intima.

O. T. SCHULTZ.

TETANUS BACILLI IN THE CARDIAC BLOOD AND SPLEEN. W. PIRINGER, Zentralbl. f. Bakt. (Abt. 1) **141**:375, 1938.

Piringer reports a case of acute tetanus in which the tetanus bacillus was recovered on two occasions from the wound before death and from the cardiac blood and spleen after death. He emphasizes the importance of more frequent efforts to cultivate tetanus bacilli from the blood in wound infections and the probable importance of serum therapy in preventing tetanus-bacillemia.

PAUL R. CANNON.

Immunology

HUMAN INFLUENZA IN SWINE. R. E. SHOPE, J. Exper. Med. **67**:739, 1938.

Antibodies capable of neutralizing human influenza virus were present in the serum of old swine on two New Jersey institutional farms but absent from the serum of young swine on the same farms. The old animals had lived through the winter of 1936-1937, in which outbreaks of disease of the upper part of the respiratory tract were prevalent among the human inmates of the two institutions, while the young swine studied were born long after these outbreaks. It is believed that the swine the serum of which neutralized human influenza virus had undergone an unrecognized infection with human influenza virus, acquired from man. The possible bearing of these observations on the theory that swine influenza was originally of human origin is discussed.

FROM AUTHOR'S SUMMARY.

IMMUNIZATION WITH ANTIGEN FROM PNEUMOCOCCI. R. J. DUBOS, J. Exper. Med. **67**:799, 1938.

Pneumococci killed by acetic acid at p_H 4.2 and then allowed to become gram-negative at p_H 7 under conditions such that no cellular distintegration takes place release in solution small amounts of a substance which is precipitable by acetic acid and soluble at a neutral reaction. This soluble fraction injected into rabbits by the intravenous route causes production of antibodies which afford definite protection to mice infected with virulent pneumococci of types I, II and III. Other types were not tried. White mice immunized with this soluble antigen exhibit some active immunity to virulent pneumococci, but the results have been very irregular so far. Soluble fractions, similar in properties and with apparently the same immunizing action, have been obtained from both virulent (S) and avirulent (R) pneumococci.

FROM AUTHOR'S SUMMARY.

QUANTITATIVE PROTECTION IN MONKEY MALARIA. L. T. COGGESHALL and M. D. EATON, J. Exper. Med. **68**:29, 1938.

The minimal infective dose of Plasmodium knowlesi for rhesus monkeys was found to be between 1 and 10 parasites when injected intraperitoneally. As the dose of parasites is increased, the length of time prior to the appearance of circulating parasites is decreased. However, the severity of the infection, once it is established, is independent of the initial dose of parasites. In experimental passive protection a quantitative relationship was demonstrated between the number of parasites in the inoculum and the effective amount of immune serum given at the time of infection and in equal doses daily for nine days thereafter: The smaller the inoculum the less was the quantity of immune serum required to prevent the death of the animal. In experimental protection, when relatively large amounts of immune serums and small numbers of parasites were used, infection was prevented.

FROM AUTHORS' SUMMARY.

BACTERICIDAL ACTION OF PROSTATIC FLUID IN DOGS. G. P. YOUMANS, J. LIEBLING and R. Y. LYMAN, J. Infect. Dis. **63**:117, 1938.

Prostatic fluid was collected aseptically from normal dogs and tested for germicidal action on colon bacilli, staphylococci (Staph. aureus), streptococci and gonococci. Marked germicidal power was demonstrated for the colon bacilli, the staphylococci and the streptococci and very slight germicidal power for the Torrey strain of the gonococci. Different samples of prostatic fluid varied considerably in germicidal action. The samples were tested for complement and lysozyme, and neither of these factors could be demonstrated. Adsorption with either B. coli or Staph. aureus removed the germicidal agent, which was apparently nonspecific. Heating at 50 C. for thirty minutes slightly reduced the germicidal power; heating at 55 C. reduced the germicidal power over 50 per cent, and heating at 60 C. for thirty minutes completely destroyed it. The prostatic fluid could be kept in the ice box for over three months without loss of potency. As tested, the germicidal power of prostatic fluid from a dog was far greater than that of blood serum taken from the same animal.

The results give no indication of the nature of the germicidal substance present in normal prostatic fluid, but the active factor does not appear to be similar to the factors that have been found to be responsible for germicidal effects in other body fluids and excretions.

FROM AUTHORS' SUMMARY.

SEROLOGIC PROPERTIES OF A POLYSACCHARIDE FROM THE MUCOID SUBSTANCE OF HEMOLYTIC STREPTOCOCCI. H. LOEWENTHAL, Brit. J. Exper. Path. **19**:164, 1938.

From hemolytic streptococci in the mucoid phase and also from type III pneumococci a substance can be isolated which has nature of a polysaccharide. Its serologic activity can be demonstrated only with serum produced by the use of cautiously killed suspensions of young encapsulated cocci. It is neither type specific nor species specific, and it appears to be unconcerned with the invasive properties of streptococci. The fact that streptococci in the mucoid phase stimulate the formation of protective antibodies and precipitins for the polysaccharide from mucoid material only when in the young encapsulated state can be explained by the process of deterioration of the bacterial surface which sets in after several hours of growth.

FROM AUTHOR'S SUMMARY.

INCREASE OF THE ANTIGENIC PROPERTY OF SERUM BY ULTRAVIOLET IRRADIATION. W. STECHER, Virchows Arch. f. path. Anat. **300**:645, 1937.

The literature contains conflicting reports on the effect of ultraviolet irradiation on the antigenic property of serum. Some ascribe to this procedure an

increase in allergic activity; others claim that the serum is denatured and loses its antigenic property. In the author's experiments, when rabbits were sensitized with untreated swine serum and then given irradiated serum as the provocative dose no anaphylactic response was elicited. However, when the animals were sensitized with the irradiated serum and given the same serum in the provocative injection 3 animals died in anaphylactic shock. The animals that survived revealed marked hyperergic inflammatory reactions. The author's findings are based on a small number of animals, 5 in each of the two experiments. O. T. SCHULTZ.

PREPARATION OF TYPHOID VACCINE WITH MERCURIC CHLORIDE. D. BARTOS and J. BUCHGRABER, Zentralbl. f. Bakt. (Abt. 1) **141**:355, 1938.

A 4 per cent solution of mercury bichloride kills typhoid and paratyphoid bacilli more rapidly and produces a vaccine less toxic than the one prepared by the use of heat. Agglutinin produced by such a vaccine appeared earlier and in higher titer than that obtained by injecting comparable amounts of heat-killed vaccine. When injected into human subjects, the mercury bichloride vaccine caused a less severe reaction both locally and generally than did the heated vaccine.

PAUL R. CANNON.

QUANTITATIVE DETERMINATIONS OF THE FRACTIONS OF COMPLEMENT. A. HEGEDUES and H. GREINER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **92**:1, 1938.

Practical methods for the separation of the four fractions of complement are presented. The titer of complement depends on the action of that fraction of which there is the least amount. The fractions cannot replace each other; i. e., an abundance of one will not compensate for the deficiency of another. The quantities of the fractions differ in different species. The serum of the guinea pig has the highest complementary action, then follow in the order of their respective titers the serums of: rat, man, hog, dog and rabbit. Man has a great deal of the thermostable fourth fraction (inactivated by ammonium hydroxide) and very little of the thermolabile albumin fraction, of which a great deal is present in the serum of the guinea pig. The thermostable third fraction (inactivated by cobra venom and by yeast) is particularly abundant in the serum of the hog and the thermolabile globulin fraction in the serums of cattle, horse, sheep and squirrel.

The serums of the four last-mentioned species showed a failure of complementary action, which was due to deficiency in (only traces) or complete absence of all the fractions except the globulin fraction. I. DAVIDSOHN.

USE OF IMMUNE SERUM IN THE TREATMENT OF ANTHRAX IN BULGARIA. P. KOSCHUCHAROFF, Ztschr. f. Immunitätsforsch. u. exper. Therap. **92**:53, 1938.

Among a population of about 6,000,000, there occur from 800 to 1,100 cases of anthrax. From 2,000 to 3,000 cases occur in domestic animals. The infection is contracted almost without exception by contact with diseased animals. The report is based on 521 cases in man. In each case the malignant pustule was very characteristic, and bacteriologic studies were superfluous. In most cases it was located on the exposed parts (face, arms, legs), but no part of the body was free. The mortality was greatest among those who had the primary lesion on the face. After a historical review of surgical and chemical therapy, the author presents the great successes of serum therapy. The administration of large doses led to improvement in results, the mortality dropping from 33 per cent in 1912, with average doses of from 30 to 150 cc., to 3 per cent in 1935 and 1936, with larger average doses, reaching in some cases 1,500 cc. The methods of producing

immune serum are reviewed. It is emphasized that no satisfactory method of standardization of anthrax immune serum is available and that normal serum is often just as efficacious as immune serum. I. DAVIDSOHN.

BLOOD GROUPS IN NOMADS OF ASIA MINOR. S. IRMAK, Ztschr. f. Immunitätsforsch. u. exper. Therap. **92**:74, 1938.

In Anatolia there are about 500,000 nomads of pure Turkish racial stock known as Yürük. They do not intermarry with the sessile population. A determination of the blood groups among 400 of them revealed the following distribution: O, 51.3 per cent; A, 40.5 per cent; B, 6.2 per cent, and AB, 2 per cent. The biochemical index was 5.2 per cent. This distribution is strikingly different from the old finding of Hirszfeld. The discrepancy is explained by the fact that Hirszfeld studied Turkish soldiers of different racial stocks. From his observations, Irmak draws far reaching conclusions concerning the validity of Bernstein's idea of the origin of the factor B and concerning the anthropologic relations between the Turks and the so-called Alpine races. I. DAVIDSOHN.

Tumors

PROGNOSTIC SIGNIFICANCE OF INTRACELLULAR MUCICARMINOPHILIC MATERIAL IN CARCINOMA OF THE FEMALE BREAST. V. K. FRANTZ, Am. J. Cancer **33**:167, 1938.

One hundred and thirty cases of operable carcinoma of the female breast have been analyzed after radical mastectomy according to the presence or absence of axillary metastases, the presence or absence of intracellular mucicarminophilic material and the grade of tumor, according to the plan submitted by Haagensen. The follow-up on the series includes 97.1 per cent for a five to nine year period. The mucicarminophilic material is found in all grades, but the number of cases in which it is found decreases with increase in histologic evidence of malignancy. The relative amount of the material is found to be related to the length of life after operation. The evidence presented indicates that the presence or absence of mucicarminophilic material provides additional information for estimating the malignancy of tumors of grade 2. It is pointed out that no histologic character yet found carries nearly the weight in prognosis that is carried by well known clinical findings, such as age, extent of local disease and presence of metastases. Moreover, there are probably many other factors of which there is little knowledge at present which determine the outcome. FROM AUTHOR'S SUMMARY.

MALIGNANT TUMORS IN DOGS. H. B. RUGGUCK and R. A. WILLIS, Am. J. Cancer **33**:205, 1938.

Nine selected malignant tumors in dogs are described, and their characters compared with the corresponding tumors of man. The tumors described comprised carcinoma of the tonsil 2 cases, carcinoma of the cardia of the stomach, carcinoma of the pancreas, carcinoma of the prostate, carcinoma of sweat glands, cystic and papillary carcinoma of the breast, mixed adenocarcinomatous and osteosarcomatous tumor of the thyroid, and primary sarcoma of the heart.

FROM AUTHORS' SUMMARY.

CANCER OF THE ALIMENTARY TRACT IN MICE. H. G. WELLS, M. SLYE and H. F. HOLMES, Am. J. Cancer **33**:223, 1938.

This article is an extension of a similar report made twenty years ago. It emphasizes again the striking infrequency of cancer in the alimentary tracts of animals of all species other than man, whose digestive canal is the site of approxi-

mately half of all carcinoma occurring in man. In 142,000 mice of the Slye stock, all dying of natural causes, without experimental manipulation, most of them of cancer age, only 15 primary malignant neoplasms of the stomach have been found, 8 being squamous cell carcinoma of the cardia, 3 adenocarcinoma of the pylorus, 3 apparently a benign epithelial growth, and 1 primary sarcoma. In the literature the authors could find reports of but 7 other cases of gastric cancer in mice. There were 19 primary intestinal neoplasms, of which 11 had arisen in prolapsed rectums: 6 were squamous cell carcinoma, 2 adenocarcinoma and 3 sarcoma in mice of a sarcoma strain, and only 1 was adenocarcinoma. There was also 1 instance of squamous cell carcinoma of the anus. In the literature only 6 other cases of cancer in the intestines of mice could be found recorded. Not a single neoplasm of the esophagus was found in these 142,000 mice, which had produced many thousands of other tumors, and the authors could find in the literature no record of a cancer of the esophagus in mice. The literature of cancer of the alimentary canal in animals is reviewed briefly, and the incidence contrasted with that in man.

FROM AUTHORS' SUMMARY.

ACTION OF X-RAYS ON EGG FRAGMENTS. P. S. HENSHAW, Am. J. Cancer **33**:258, 1938.

Experiments have been carried out to determine whether a certain radiobiologic effect in cells results from changes produced in the nucleus or in some other part of the irradiated cells. The effect referred to was a delay in cell division. The test material used was eggs of Arbacia punctulata. Nucleated and non-nucleated egg fragments were obtained by the centrifuge method, both of which will undergo fertilization and development. These were irradiated and inseminated, and the moment when 50 per cent had divided was determined. It was found that for a given dose of radiation an appreciable delay was produced in whole eggs and nucleated fragments of about the same extent in each, whereas no delay was produced in the non-nucleated fragments. Thus, since there is direct correlation between the presence of a nucleus and the manifestation of the irradiation effect and since changes occurring in parts exclusive of the nucleus produce no observable effect, it appears that the slowing in cell division brought about in the eggs of Arbacia punctulata by roentgen rays is due to changes produced directly in the nucleus by the radiation.

FROM AUTHOR'S SUMMARY.

A CLINICOPATHOLOGIC STUDY OF ASTROCYTOMA. R. W. WAGGONER and K. LÖWENBERG, Arch. Neurol. & Psychiat **38**:1208, 1937.

Waggoner and Löwenberg state that astrocytoma involves the hemispheres as well as the brain stem and very often grows bilaterally, in contrast to other types of glioma, the growth of which is usually unilateral. Sections of the whole brain harboring astrocytoma were stained by the method of Weigert, and the areas of demyelination were traced. On the location of the nucleus of the tumor depends the further mode of invasion. For instance, when the nucleus of the tumor is in the corpus callosum, the growth is bilateral and invades the frontal lobes and the anterior parts of the parietal lobes. If the nucleus of the tumor is in the splenium of the corpus callosum, the growth invades the temporal and occipital lobes and both basal ganglions. Unilateral astrocytoma seldom invades the corpus callosum; it preferably invades the pyramidal tract.

GEORGE B. HASSIN.

THE FAMILIAL ASPECTS OF CANCER. H. L. LOMBARD, New England J. Med. **218**:711, 1938.

All the methods of study used point toward some hereditary tendency to cancer. They do not indicate whether all cancer is hereditary. The statistical means at one's disposal make it impossible to investigate this point. A general hereditary

susceptibility may or may not be present. This study indicates merely that persons among whose immediate relatives cancer has occurred have a slightly greater chance of contracting the disease than has the remainder of the population. The difference between the two groups is not sufficient to cause undue worry, but indicates without any question that further statistical studies on this problem are desirable.

FROM AUTHOR'S SUMMARY.

THE CHEMICAL COMPOSITION OF THE ACTIVE AGENT OF THE ROUS SARCOMA No. 1. A. POLLARD, Brit. J. Exper. Path. **19**:124, 1938.

The tumor-producing agent of Rous sarcoma 1 can be deposited from cell-free extracts by means of the Sharples centrifuge. The resuspended deposit can be purified further by fractional centrifugation. The product obtained consists mainly of protein, with a variable amount of lipoid material. Preparations of lipoid-extracted tumor desiccates contain much smaller amounts of lipoid but retain their tumor-producing activity.

FROM AUTHOR'S SUMMARY.

SPECIFIC COMPLEMENT-FIXATION WITH THE VIRUS OF SHOPE'S FIBROMA. C. E. VAN ROOYEN, Brit. J. Exper. Path. **19**:156, 1938.

Rabbits immunized with either the IA or OA strain of the virus of Shope's fibroma in the living state had complement-fixing as well as virus-neutralizing properties in their serums. The complement fixation reactions afforded no basis of distinguishing between the IA and OA strains. Complement-fixing antibodies appeared about two to three weeks after injection of the virus. They usually disappeared about eight weeks after the last injection of virus. Virus heated to 55 C. for one hour retained its antigenic properties, giving rise to complement-fixing and neutralizing antibodies. Nothwithstanding repeated doses of living (or dead) virus, no complement-fixing antibodies appeared in the serum of certain rabbits. The serum of such animals, however, showed a powerful virus-neutralizing effect. Therefore, though all serums yielding complement fixation also neutralized the virus, it could not be said that all serums showing neutralization of the virus showed the presence of complement-fixing antibodies.

FROM AUTHOR'S SUMMARY.

GIANT CELL TUMOR OF BONE. E. K. DAWSON, J. R. M. INNESS and W. F. HARVEY, Edinburgh M. J. **45**:491, 1938.

Giant cell tumor of bone is a neoplastic growth, probably originating with trauma and hemorrhage and developing as an osteogenic reaction and proliferation, which may slowly progress and ultimately regress, but which in rare cases acquires the characters of osteogenic sarcoma. It may be classed as a type of new growth intermediate between purely reactive tissue and true blastoma.

FROM AUTHORS' SUMMARY.

MICROMANIPULATION AND MICRODISSECTION OF THE INCLUSION BODY OF MOLLUSCUM CONTAGIOSUM. C. E. VAN ROYEN, J. Path. & Bact. **46**:425, 1938.

Fresh material obtained in 10 cases of human molluscum contagiosum has been stained intra vitam with brilliant cresyl blue and subjected to microdissection. The inclusion body of molluscum contagiosum has been successfully removed from the infected epithelial cell. It has also been replaced in the same cell without injuring the latter. The removal of the inclusion body leaves a large cavity in the cell. The cytoplasm does not flow into this space, the cavity being apparently lined by a localized condensed layer of cytoplasm. The boundaries of this cavity have been defined by injecting india ink into it or alternatively by introducing india ink into the surrounding cytoplasm. The inclusion body prob-

ably grows from a more minute form of the virus. It is not formed by desiccation or by fusion of any intracytoplasmic constituents of the cell. It is suggested that the large oval or pear-shaped form of the inclusion body represents a later stage of development than the smaller spherical one. The large pear-shaped inclusion bodies have been shown to possess a definite outer membrane, which is thinnest at the conical pole. These observations have been established by demonstrating the tensile strength of the membrane on "palpation" with the point of a microneedle. The weakness of the conical segment has also been revealed by transfixing the inclusion body between two needles and exerting traction on its walls. The elementary virus bodies contained within the inclusion body of molluscum contagiosum have been shown to be supported in a gelatinous mucoid substance.

FROM AUTHOR'S SUMMARY.

IMMUNITY AGAINST CARCINOMA IN THE RABBIT. A. BESREDKA and L. GROSS, Ann. Inst. Pasteur **60**:5, 1938.

Brown-Pearce carcinoma was usually fatal when injected into the testicle, brain or anterior chamber of the eye of the rabbit. The mortality after subcutaneous injection was lower. Intracutaneous inoculation produced a benign tumor, which was ultimately resorbed and which rendered the animal immune. Rabbits naturally refractive have never been found.

The carcinomatous material was attenuated so that it did not produce tumors by storage at 0 C. for twenty-one days or at 37 C. for from two to three days, by addition of bile or glycerin or by freezing and thawing. Rabbits treated with attenuated material were not immune to active carcinoma.

Intracutaneous inoculation of unattenuated material uniformly resulted in solid and lasting immunity. Tumors could not be produced in immune rabbits regardless of the size of the dose or of the route of administration. This immunity was entirely cellular and could not be transmitted passively. Serum from immune animals in doses of 50 cc. had no specific effect whether given before or after the introduction of the epithelioma. Experiments in parabiosis also failed to show any evidence of passive transfer.

J. B. GUNNISON.

BASILAR AND SACRAL CHORDOMA. H. VON BRAITENBERG, Frankfurt. Ztschr. f. Path. **50**:509, 1937.

The author reviews the recent literature and cites the classification of chordoma according to Coenen and Linck. Five tumors of this type are then presented. Three of these were basilar chordoma. Two were benign. The third was malignant and composed chiefly of the large vesicular cells characteristic of chordoma, with intracellular spaces containing much mucoid material. Two of the tumors were malignant sacral chordoma with invasion of the surrounding bones. The author believes that chordoma belongs to the dysontogenetic types of tumor, and that it arises from remaining chorda complexes in the perivertebral tissue. In chordoma are found cells typical of all the different stages of development of the chorda dorsalis, the early cell types being present chiefly in the malignant forms. The amount of mucoid material is larger in malignant than in benign chordoma. This material is thought to be the product of degenerated cells. Also, glycogen, which is present in the cells of the chorda dorsalis, is found in the tumor cells.

ANNEMARIE STRAUSS.

CULTIVATION OF ADULT TISSUES IN HUMAN SERUM. R. TASZKAN, Virchows Arch. f. path. Anat. **299**:106, 1937.

It was found possible to cultivate adult human and animal tissues in human serum in the absence of chick embryo extract. Chicken plasma and human serum in equal quantities of 0.5 cm. were used, and the tissue fragment was implanted.

After coagulation of the medium, 1 cc. of Ringer's solution was added. After two days of incubation, the liquid was pipetted off and replaced by human serum diluted 1 : 4 with Ringer's solution. Thereafter the liquid portion was changed every five days. By this procedure it was possible to cultivate connective tissue of the spleen, heart, kidney, placenta and liver and epithelium of the kidney and liver. The growth-promoting factor is held to be derived from cells in which the membrane has been injured and into which unassimilable substances have penetrated. The injury to the cells is overcome by growth. The serum of pregnant women and of persons with malignant tumors supported growth better than did normal human serum. The disintegration of tissue within the organism leads to increased proteolysis; the proteolysins are active only against the denatured proteins of the organism from which they are derived.

O. T. SCHULTZ.

THE SO-CALLED ASTROCYTOMA OF THE CEREBELLUM. H. BERGSTRAND, Virchows Arch. f. path. Anat. **299**:725, 1937.

Cushing noted that astrocytoma of the cerebellum differs from cerebral astrocytoma in the fact that it occurs predominantly in children and young adults and in the fact that a much better prognosis may be given if the tumor is removed surgically. In 1932 Bergstrand reported the results of a study of 11 examples of this tumor and concluded that the term "astrocytoma" is a misnomer. He now reports on 45 additional tumors of this group. Of the 56 tumors, 18 occurred in the first decade of life, 15 in the second and 16 in the third. So-called astrocytoma may involve the pia as well as the parenchyma of the cerebellum, in which case the pia contains heterotopic tissue. The cerebellar tissue adjacent to the tumor reveals malformation. The stratum granulosum is reduced, Purkinje cells are absent, and on the surface of the molecular layer is an added zone of neurofibrils and glia by means of which the tumor is attached to the pia. The tumor itself represents congenital maldevelopment. "Astrocytoma" is an improper name for it because the growth consists of embryonic glia cells, neuroblasts, axis-cylinders and medullated nerve fibers. It should be termed glioneuroblastoma. Areas of the tissue may become differentiated, fibrous and very hard. Because the tumor is congenital, few of its victims reach the age of 25 years unless the tumor has been surgically removed.

O. T. SCHULTZ.

EPITHELIAL CHANGES IN THE FEMALE BREAST IN RELATION TO AGE AND CANCER. B. KORPÁSSY, Virchows Arch. f. path. Anat. **299**:793, 1937.

Presumably normal breasts obtained at necropsy from 300 women aged 13 to 89 years were examined histologically with special reference to epithelial proliferation. In each instance both breasts were examined. Dilatation of small ducts into cysts was so common as to be almost physiologic, being observed in two thirds of the cases. Duct dilatation with epithelial proliferation occurred in 48.7 per cent of all the cases, and the incidence as well as the extent increased progressively with age, the incidence reaching a maximum of 75.5 per cent in women over 70 years of age. The proliferated epithelium was of the small cuboidal type that normally lines the ducts or of the tall pale apocrine type. Although the degree and frequency of epithelial proliferation increased with age, no changes suggestive of invasion or early malignant growth were observed. In a woman aged 80 years histologic examination revealed a small carcinoma in each breast; neither growth was associated with other epithelial proliferation. In a woman aged 64 a pea-sized carcinoma of the breast was discovered; in the involved breast there was proliferation of duct epithelium. Statistical analysis of the author's material in comparison with a series of carcinomatous breasts established that in cancerous and noncancerous breasts there is a progressive increase in degree and frequency of epithelial proliferation with advancing years and that epithelial proliferation is more frequent in breasts with cancer than in those without. Although

the author's material yielded no evidence of direct transformation of the epithelial proliferation of cystic disease of the breast to carcinoma, he looks on the epithelial proliferation as the last stage before cancer.

O. T. SCHULTZ.

THE ASSOCIATION OF PACHYMENINGITIS WITH TUMORS. E. LASS, Zentralbl. f. allg. Path. u. path. Anat. **69**:404, 1938.

In a series of 46 bodies with pachymeningitis haemorrhagica interna there were 6 which had cellular nodules attached to the dura. These nodules seemed to arise from the inner aspect of the dura. They protruded above the surface of the thin young membranes and were buried deeply in the thick old membranes. The cells of the protrusions resembled those coating the dura, and most clumps contained numerous psammoma bodies. There seemed to be no relation between the occurrence of such nodules and the age of the patient or the thickness or extent of the pachymeningitis.

The richest nodular hyperplasia was encountered in the body of a woman 85 years old who had marked arteriosclerosis. Her brain was altered by softening. The dura of the right middle and posterior cranial fossae was covered by pachymeningeal membrane and contained linseed-sized firm nodules. As a differential diagnostic aid, Lass cites the case of a woman whose dura was altered extensively by metastases from a cancer of the breast. The nodules encountered were veins filled with tumor cells.

The possibility is advanced that nodular hyperplasia is related to true neoplastic growth, such as meningioma. Hyperplasia occurs also in sites unassociated with pachymeningeal membranes and possibly is related to some vascular disturbance. It is known that new capillary formation occurs on the inside of the dura when the veins on the outside are obstructed. New vessel formation does not occur when the veins on the inside of the dura are occluded.

GEORGE J. RUKSTINAT.

Society Transactions

PATHOLOGICAL SOCIETY OF PHILADELPHIA

BAXTER L. CRAWFORD, *President*

Regular Meeting, May 12, 1938

HERBERT L. RATCLIFFE, *Secretary*

OSSEOUS CHANGES IN MAN FOLLOWING PROLONGED INGESTION OF FLUORIDES: REPORT OF A CASE. JOHN T. BAUER.

Chronic fluoride poisoning in man has been manifest in two forms: (1) mottled enamel, or dental fluorosis and (2) osteosclerosis. An instance of the latter occurred in a Negro laborer who for eighteen years had handled finely ground rock phosphate, a sample of which contained 3.88 per cent fluorine. He was admitted to the medical service of Dr. R. H. Farley at the Pennsylvania Hospital because of syphilitic aortitis. Roentgenologically the osseous changes were increased density, loss of detail despite increased exposure and lack of normal sharpness along the tendinous attachments, with formation of osteophytes and exostoses. These changes were greatest in the vertebrae, ribs and sternum and least in the long bones of the extremities. Gross examination of the bones confirmed the roentgenologic report of osteophytes, exostoses and roughening of the muscular attachments. Also noted were: (*a*) chalky white patches on the surfaces, (*b*) plaques of thin cortical bone and (*c*) increased thickness of the cortex of the ribs and, to a less extent, of the bones of the extremities. Microscopically, the cortex of the ribs encroached on the marrow spaces, but amorphous granules were not observed. Determinations of the specific gravity of the bones indicated that they were less dense than normal; hence the increased opacity to irradiation was thought to be due to increased formation of bone. Chemically, the bones had from ten to twenty times the normal fluorine content. The bones with the greatest amounts had given the greatest differences roentgenologically in life. Analyses for ash, calcium, phosphorus and carbon dioxide gave normal values.

Clinically and pathologically the patient did not have anemia or other changes which could be ascribed to the prolonged ingestion of fluorides. From the standpoints of the roentgenologist (Bishop, P. A.: *Am. J. Roentgenol.* **35**:577, 1936), the pathologist (Bauer, J. T.; Bishop, P. A., and Wolff, W. A.: *Bull. Ayer Clin. Lab., Pennsylvania Hosp.* **3**:67, 1937) and the chemist (Wolff, W. A., and Kerr, E. G.: *Am. J. M. Sc.* **195**:493, 1938), the observations on this case indicate that it agrees in general with the two others similarly studied and reviewed by Roholm (Fluorine Intoxication, London, H. K. Lewis & Co., Ltd., 1937).

NOTES ON A FORM OF RENAL DISEASE OF THE DOG. H. M. MARTIN.

Of domestic animals, the dog seems to be the most commonly and most seriously affected by renal disease. And perhaps the most frequent and important lesion of the kidney of the dog is a kind of interstitial nephritis which often appears as a sequel to distemper, especially when this condition is complicated by pneumonia. Other acute infections also may be considered as causative factors.

The acute stages of interstitial nephritis are not often seen at necropsies, probably because the animals usually survive these stages and the disease progresses to the subacute or chronic phases with scar formation. Thus the "inflammatory shrunken kidney" of the dog is believed to be identical in most instances with the late stages of interstitial nephritis.

In the acute phases of this disease the capsular surface of the kidney is elevated by well circumscribed grayish or yellow-gray foci of variable dimensions. These are more or less closely placed over the organ. On the cut surface similar infiltrations occur as small circumscribed nodules or wide irregular areas obscuring the usual markings and causing widening of the cortex so that it comes to approximate the width of the medulla.

The histologic changes vary with the gross appearances, but in all a constant feature is more or less extensive infiltration of the interstitial tissues by cells of the lymphocyte series, chiefly large forms, with small numbers of polymorphonuclear leukocytes. These cells accumulate principally in the cortex, but in severe cases the medulla is involved also. Occasionally the medulla alone is infiltrated. Small blood vessels also contain increased numbers of lymphocytes. The tubules and glomeruli are essentially unchanged at this time.

These acute phases of the disease apparently are followed by scarring with the result that the kidney may come to have an appearance resembling that which follows multiple infarction or pyelonephritis; or scar formation may be diffuse, and a small granular, tough, leathery organ result. With these changes the cortex is narrowed greatly.

The microscopic appearance of such organs may be similar in certain respects to that due to chronic glomerulonephritis, and differentiation may be difficult. However, glomerular changes in this instance appear to be part of the general interstitial disease and affect first Bowman's capsule with secondary disease of the capillary tuft. Calcification of the glomerular capsule with fibrosis and calcification of the tuft may occur, or glomeruli may appear hypertrophic. An interstitial infiltrate of lymphocytes may be seen about the scarred areas, suggesting progressive disease.

The clinical signs of interstitial lymphocytic nephritis vary. Often the first evidence of disease is ulcerative stomatitis. There may be also marked visual disturbances or ascites or hydrothorax. Usually anemia is present. Probably the most common terminal change is severe hemorrhagic gastroenteritis.

That renal function is impaired may be shown by an analysis of the blood. The specific gravity of the urine is low, albumin is present in variable amounts, and casts, leukocytes and erythrocytes are often found.

LEUKOSIS OF FOWLS. E. L. STUBBS.

"Leukosis" is a term used to include the leukemia as well as the leukemia-like diseases of fowls. Several different types of leukosis have been described, some of which are transmissible (a fact which facilitates study of these types) while others cannot be transmitted and are not well understood.

Transmissible types are readily transferred from diseased to normal chickens by injections of blood, organ emulsions or filtrates. The causative agent is more active in the blood cells than in the plasma and filtrates. Transmission succeeds best with injection of whole blood. In tests made thus far the causative agent has resisted drying from the frozen state for at least nine hundred and thirty-two days.

The period of incubation varies from one week to four months. The younger the chickens the more susceptible they are as measured by the period of incubation and the mortality. Natural methods of spread are not well understood. Mosquitoes and mites have been tried as vectors of this disease, without success.

There have been reported strains of leukosis that produce leukemia when injected intravenously and tumors when injected locally. Apparently such strains stimulate cells of more than one type to unrestricted growth. Mixed strains may produce a wide variety of changes. Some strains appear pure and produce results of only one type: the stimulation of the hemopoietic tissues to abnormal activity.

TUMORS OF THE NERVE SHEATHS IN FISH. BALDUIN LUCKÉ.

The tumors reported here closely resemble the complex group of human neoplasms arising from nerves. They were found in 39 fish belonging to three

species of snappers. Usually but one growth was present, though occasionally several were noted. The tumors were firm and resilient and usually had a white cut surface. Histologically they showed considerable variation in structure. They usually had a distinctly fasciculated makeup, and the cells were arranged in interlacing bands or whorls. In many tumors the oval nuclei were grouped in more or less parallel rows or palisades, between which were dense masses of fibrils.

The relative ease with which these tumors may be secured in tropical waters renders them favorable material for the study of an important group of neoplasms.

NEOPLASTIC DISEASE OF THE PANCREAS IN REPTILES. HERBERT L. RATCLIFFE.

Some time ago, in the course of autopsies at the Zoological Garden, enlargement of the pancreas of a pine snake was noted. In sections of this organ much of the normal tissue seemed to have been replaced by neoplastic epithelium (Ratcliffe, H. L.: *Am. J. Cancer* **24**:78, 1935). As a result of this observation histologic study of the pancreas in reptiles became a routine practice, and apparent tumor formation has proved to be relatively common.

Thus far the disease has been limited to snakes, the greater number of which have represented North American species. In 72 pancreases from these animals the gross appearances have not been informative, but sections revealed in 15 extensive destruction of the parenchyma by tissue that resembled carcinoma. Focal changes that suggested early stages of tumor growth were found in 9 other specimens.

According to my present interpretations, the earliest stages of the disease are small areas within the organ that are occupied by abnormal branching ducts, loosely arranged and pale-staining, among which are fragments of glandular tissue. These ducts are composed of irregular, indistinctly outlined flattened epithelial cells, the nuclei of which are variable in size but larger than normal.

In more advanced foci parenchyma cells are not recognizable, the ducts are more prominent, and their cells less regularly arranged, often forming multilayers about distorted lumens. Groups of these elements usually lie in loose formation with variable amounts of fibrous tissue between.

Apparently the process extends from the smaller foci to involve the whole organ, glandular tissue being replaced more or less completely by either an irregular network of ductlike structures which are supported by stroma of varying density or more compactly massed elements that resemble acini.

There appear to be two possible explanations for these lesions: Either the whole process represents primary hyperplasia of the ducts with secondary damage of the parenchyma, or the parenchyma is damaged primarily and the ductal hyperplasia is an attempt at regeneration. To me the first suggestion seems the more suitable.

NEW ENGLAND PATHOLOGICAL SOCIETY

CHARLES BRANCH, *President*

Regular Meeting, Oct. 21, 1938

GRANVILLE A. BENNETT, *Secretary*

ETIOLOGIC FACTORS IN THE DEVELOPMENT OF SILICOSIS. LEROY U. GARDNER.

While it is generally agreed that of all the inorganic dusts only free silica and asbestos are particularly harmful, there is still uncertainty about what constitutes a hazardous concentration of silica in the atmosphere. Experimental observations and observations in the field indicate that the atmospheric concentration of silica regarded theoretically as hazardous has not invariably been

associated with disease and that the amount of pulmonary reaction is not directly proportional to the quantity of silica in the rock creating a particular dust. There are two sets of factors whose action tends to retard the anticipated effects of silica; one set, acting in the atmosphere, brings about flocculation of silica with a reduction in the amount inhaled, and another, acting inside the body, retards the ordinary irritating effects of silica on the tissues. It is postulated that the latter retardation is the result of coating the grains of silica in various ways so that effective contact of silica with tissues is interrupted.

Examples of atmospheric reduction in silica from the point of dust generation to the breathing zone were shown, and the chemical and histologic effects on experimental animals were demonstrated. Evidence for the belief in atmospheric flocculation was presented.

The case for inhibitory action inside the body is based on a series of experimental observations. First, all crystalline and cryptocrystalline forms of silica excite characteristic reactions, and the rate of tissue response is inversely proportional to the particle size of the silica. The latter observation indicates a direct relation between the extent of the exposed mineral surface and the irritating properties of the mineral. Second, various silicates and nonsiliceous minerals do not produce such a reaction; in fact, most of them are essentially inert regardless of their silica content or physical properties. Third, the injection of different natural and artificial mixtures of free silica with silicates and nonsiliceous minerals does not produce reactions proportional to the amount of free silica. If the mixtures contain enough free silica, a modified type of silicosis ultimately develops but at a much delayed rate. In other words, time rather than dose of silica is the determining factor. Finally, experiments with metallic aluminum and the hydroxides of aluminum and iron form the basis for the belief in a protective coating of the silica particles.

DISCUSSION

R. Z. SCHULZ: There are several questions which may be raised in connection with silicosis, its progress and the complications which may arise. The important factors in the production of the silicotic lesion have been emphasized by Dr. Gardner. Substances which may inhibit or delay the production of these lesions are of industrial and clinical importance. Erwin has advocated the use of aluminum dust as such an inhibitory substance. Limestone dust has also been used. What is Dr. Gardner's opinion concerning the role of such substances in delaying the production of the silicotic lesion? The fact that certain substances can delay the production of the lesions is also of importance to those who attempt to evaluate the hazards arising from exposure to dust in industry.

Another question I should like to ask has to do with the relation between silicosis and tuberculosis. Concerning this matter, some work has been done which indicates that when silica is added to artificial mediums for the cultivation of tubercle bacilli it has a stimulating effect on the growth of these organisms. I should like to have Dr. Gardner express his opinion concerning the possibility that silica acts as a stimulating factor in the development of tuberculosis in those human beings in whom both disease processes occur.

FRANCIS P. MCCARTHY: I hope Dr. Gardner will say something on the differentiation between silicosis and silicotuberculosis. It is of some interest to note that the pulmonary consolidation in silicosis is concentrated in the lower lobes of the lungs.

Figures pertaining to the incidence of silicosis and silicotuberculosis in patients examined at the Braintree Tuberculosis Hospital and the Quincy City Hospital indicate that silicotuberculosis is more prevalent in an older age group than is tuberculosis alone.

A questioner whose name was not obtained asked: Will Dr. Gardner say something more about inhibitor minerals?

Another question was: Apropos of the question on inhibitors, are there substances that will accelerate the progress of silicosis?

DR. LEROY U. GARDNER: In reply to a question as to the injury produced by spun glass: Injection of such material into the ear veins of rabbits produced no more reaction than the natural silicates previously described. It was therefore concluded that this material should not be considered as a dust likely to produce pulmonary fibrosis.

Several questions sought further elucidation of the action of inhibitor minerals. I believe that they merely delay the interaction between the surface of the silica particles and the body fluids; in all cases thus far observed their effect was temporary, and when observations were continued over a sufficient period silicotic fibrosis of a modified type ultimately developed. It might be possible, of course, to coat the surface of quartz grains with membranes which would never be dissolved or dislodged, and in this case permanent protection would be theoretically possible. The experiments of Kettle may be cited. He obtained no reaction to the injection of silica particles chemically coated with iron.

In discussing the possibility of substances which would accelerate the effects of silica in the tissues, doubt must be expressed as to the validity of this theory. I have not attempted to investigate such activity by experiment, but I may cite the negative evidence published by Dr. Cary McCord. He stated that in his opinion the similarity between the lesions of rapidly developing silicosis in the "soap powder cases" and those in sand blasters and workers in the Gauley Bridge tunnel pointed to an excessive concentration of comparatively pure silica in a fine state of subdivision as the probable cause of the rapidly developing disease.

All of the other questions bore on the subject of tuberculosis in association with silicosis.

In response to a question as to how to differentiate individual silicotic from tuberculous lesions, I admit the difficulty but I place much reliance on the distribution pattern of the lesions. To observe this pattern I employ large thick sections, 3 or 4 inches (7.5 or 10 cm.) in diameter, which can be studied with a low power binocular microscope. The arrangement of hyaline collagen in typical silicotic foci tends to be more uniform and presents concentric lamination, which is not found in tuberculosis. While necrosis (and even calcification) may occur in the centers of pure silicotic nodules, it is not usually associated with the leukocytic reaction. The tuberculous reaction is rarely confined to the area of nodule formation but extends into the surrounding pulmonary air spaces. In areas of conglomerate fibrosis a tuberculous complication may be manifested by cellular reaction and caseation typical of tuberculosis or sometimes merely by diffuse necrosis involving all of the preexisting silicotic reaction. In the latter case the cellular and fibrous elements lose their sharp outlines, the nuclei no longer stain, and the whole focus presents a pale ground glass appearance. Infiltration of the focus by polymorphonuclear leukocytes is usual. Stains for tubercle bacilli may or may not disclose organisms. The result of inoculation of involved tissue into guinea pigs is a more reliable criterion of infection.

Cavities may form in the centers of areas of massive fibrosis as a result of ischemic necrosis. Such cavities are different from those due to tuberculosis. Instead of being spherical or ovoid, they are generally elongated and slitlike. They do not show a wall composed of well defined layers; their margins are composed of granular débris abutting on firm, usually deeply pigmented scar tissue. They are not usually trabeculated.

In reply to a question as to the means of differentiating in the living subject between the area of massive fibrosis in simple silicosis and the lesion of tuberculosilicosis, one must state that it is often most difficult and requires prolonged observation. The simple condition, I believe, is due to excessive accumulation of silica and other dust in pulmonary tissue damaged in most cases by previous infection. Massive fibrosis due to tuberculous complication might arise either from reactivation of an old latent focus or from reinfection in a resistant silicotic subject. Since the course of such disease is extremely chronic, without symptoms of intoxication and without tubercle bacilli in the sputum for many years, the condition might readily be confused with massive fibrosis of the first

type. However, the ultimate outcome of lesions in which living tubercle bacilli have played an etiologic role is the development of typical chronic tuberculosis with all of its characteristic signs, symptoms and tissue changes. Such an outcome might ensue only years after the first recognition of an area of tuberculosilicosis in a roentgenogram.

The differentiation of the two lesions is based on repeated physical, roentgenographic and bacteriologic examinations of the subject. A progressive loss of body weight might be the first indication of activity. Inoculation of the sputum into guinea pigs or culture of the sputum might be necessary to detect the presence of a few tubercle bacilli. A negative tuberculin reaction is significant, provided a strong enough tuberculin is injected. (Ordinary doses frequently fail to produce reactions in men with such lesions.) Both kinds of lesions produce dyspnea, which may or may not incapacitate for work. Since treatment for such advanced lesions accomplishes little, patients should all be watched for the appearance of tubercle bacilli in the sputum. In this event they should, of course, be removed from contact with others and particularly from contact with any fellow workman who is unduly susceptible to tuberculosis by virtue of having silicosis.

CHARLES BRANCH, *President*

Regular Meeting, Nov. 17, 1938

GRANVILLE A. BENNETT, *Secretary*

PATHOLOGIC OBSERVATIONS ON EQUINE ENCEPHALITIS IN MAN. SIDNEY FARBER and CHARLES BRANCH.

This report concerns material from nine postmortem examinations at the Massachusetts Memorial Hospitals and five at the Children's Hospital, Boston, in the early fall of 1938. The diagnosis of equine encephalitis was made either by the identification of the virus obtained from the brain (L. D. Fothergill and J. H. Dingle; L. T. Webster and F. H. Wright) or by the results of pathologic examination. A composite picture of our observations follows:

Edema and congestion of the brain and spinal cord and generalized visceral congestion were present to a severe degree. On microscopic examination diffuse meningoencephalomyelitis was found. The changes may be regarded as of several types. First, there was widespread involvement of nerve cells ranging from early involvement of nuclei and cytoplasm to complete disappearance of these. The areas of dead and dying neurons contained cellular collections composed mainly of polymorphonuclear cells and microglial cells. The second type of change consisted in perivascular accumulations of cells. In the early stages of the disease this reaction consisted mainly of neutrophils, lymphocytes and large mononuclear cells. As the duration increased, the predominant cells were lymphocytes and large mononuclear cells, a cell picture which paralleled that in the spinal fluid. Third, there was diffuse meningitis, most marked over the base of the brain and present only to a slight degree over the cord. The meningeal reaction corresponded to that found in the perivascular spaces. One instance was noted in which the meningeal reaction over the base of the brain was severe enough to give the gross picture of acute pyogenic meningitis. The fourth type of characteristic reaction consisted in the presence of lesions in both arterioles and venules. Numerous small thrombi were present in the small vessels. In addition, many of the vessels showed complete involvement of their walls, characterized by neutrophilic infiltration and deposition of fibrin actually within the walls. Demyelination was not a prominent feature except in areas where tissue had been destroyed entirely by the inflammatory process.

The cord in most of the cases showed no involvement except for edema and congestion. Occasional evidences of early damage to nerve cells could be demonstrated. One case was noted in which the entire cord showed evidences of nerve cell destruction.

The distribution of the lesions was of some interest. The most severe involvement was found in the basal ganglions and in the brain stem. There was diffuse involvement of the cortex, although numerous small uninvolved areas could be found. The olfactory bulb was either spared or little involved. The cerebellum showed occasional slight evidences of inflammation, and these corresponded in degree of severity to those found occasionally in the cord.

This type of encephalitis falls into Greenfield's group II of polioclastic encephalitis. In this group are found encephalitis lethargica, rabies, equine encephalomyelitis, St. Louis encephalitis, Japanese encephalitis B and poliomyeloencephalitis. The picture in equine encephalitis as seen in this material corresponds closely to that described in St. Louis encephalitis and Japanese encephalitis B. Qualitatively the changes are similar to those found in encephalitis lethargica; the nearest comparison, which amounts in some cases almost to duplication, particularly after the disease process has reached the five day stage, is that to the picture in St. Louis encephalitis. Vascular lesions, which were so prominent in these instances of equine encephalitis, have not been stressed in the other forms. Except for the difference in distribution, with particular reference to the absence of involvement of the anterior horn cells in the spinal cord in this disease, the type of damage and cellular response closely corresponds to that observed in poliomyeloencephalitis. No bacteria were found in association with the early lesions, either by cultural or by staining methods.

The changes in the body consisted mainly of congestion and edema. Severe pulmonary edema was present as a terminal phenomenon in all cases. In the lungs of a patient who died forty-eight hours after the onset and from whose blood stream no organisms could be cultured, early interstitial pneumonia was found. In various organs of the body of the same patient small vessels showed scattered small thrombi.

A detailed report of the pathologic observations is being prepared.

DISCUSSION

ALLEN M. HILL (by invitation): During a period of six weeks, beginning late in August and ending early in October, there was admitted to the Children's Hospital a group of 8 patients who, by the character of their illnesses, the physical findings and the results of studies of the spinal fluid, appeared to be suffering from a severe form of encephalitis. To date 5 of these patients have died, and 3 have recovered sufficiently to be able to return home. All of those who succumbed were examined post mortem, and from 3 a virus was recovered which was identified as that of the Eastern strain of equine encephalomyelitis. A study of the serums of those who recovered, in which neutralization tests are being made, has not as yet been completed.

The ages of the patients ranged from 1 month to 7 11/12 years. Seven were infants under 18 months of age.

In none of the patients did the illness progress for a period of more than forty-eight hours before admission to the hospital. The onset was abrupt, with all the patients becoming acutely ill within three to twelve hours. Each presented on admission to the hospital two or more of the following symptoms: high fever, vomiting, coma, rigidity and convulsions. At one time or another during the illness all had one or more generalized tonic, and occasionally clonic, convulsions. Neurologic examination revealed Kernig's signs, mild cervical rigidity and semiconsciousness or coma, and in infants, tense bulging of the fontanel. In those who survived sufficiently long, a peculiar nonpitting edema, prominent in the periorbital spaces and to a lesser extent in the extremities, developed three to four days after the onset of the illness. Leukocytosis was present in all, the cell count ranging from 13,600 to 35,000, with the differential

count showing from 64 to 90 per cent polymorphonuclear leukocytes. The spinal fluid (on admission) showed from 246 to 2,000 cells, with polymorphonuclear leukocytes predominant in percentages of 60 to 100. Within a few days the spinal fluid cell count decreased, and this decrease was accompanied by a reversal in the polymorphonuclear leukocyte–mononuclear cell ratio. The protein of the spinal fluid was elevated in all (50 to 140 mg. per hundred cubic centimeters), the sugar content was normal (qualitative), and cultures gave no bacterial growth. A search for tubercle bacilli was unsuccessful.

Of the patients who died, 2 succumbed within forty-eight hours after the onset of the illness, 2 lived seven and nine days, respectively, and 1 died at the end of twenty-one days. Of the 3 children who survived, all were found to have some neurologic residual: 1, spastic right hemiplegia and partial deafness; 1, right hemiparesis and incoordination of the upper extremities, and 1, spastic diplegia. There has been some degree of improvement in all these patients since their return home.

LeRoy D. Fothergill (by invitation): The clinical features, epidemiologic aspects and mode of transmission of the Eastern and Western varieties of equine encephalomyelitis were reviewed. During the past summer an extensive epidemic of encephalomyelitis occurred among horses in southeastern Massachusetts. Encephalitis appeared in a number of human beings in the area where the horses were diseased and was shown to have been caused by the virus of the Eastern variety of equine encephalomyelitis. This virus was isolated from the brain tissue in 8 fatal cases. Its identity was established by protection tests in immunized guinea pigs and by neutralization tests with hyperimmune serums.

During this epidemic a total of about 40 probable cases in man came under observation. The mortality rate was about 65 per cent. At the present time, extensive studies are being conducted by means of neutralization tests. The serums of convalescent patients, of various contacts of the patients, of veterinarians and of various other persons, such as those suffering from minor illnesses, are being examined. Clearcut positive results are being obtained with the serums of convalescent patients. Neutralizing antibodies have not been demonstrated in the serums of family contacts or in veterinarians so far as concerns the specimens examined up to the present time. A detailed report of these studies will be published in the near future.

John H. Dingle (by invitation): I have but one or two comments to add. The virus of equine encephalomyelitis has been widely used in investigative work, not only because there is little danger of laboratory infection in man, as Dr. Fothergill pointed out, but also because the common laboratory animals are susceptible to it. In addition, experimental infections have been produced by Remlinger and Bailly and by others in various avian species. No such infection, however, has been produced in the chicken.

In 1933 Giltner and Shahan produced infection in pigeons with the Western type of the virus. These workers, as well as Ten Broeck, Hurst and Traub, pointed out the possibility that birds were host reservoirs.

During the recent epidemic here the death of unusual numbers of pigeons was noted by dealers and residents in the region of the epidemic and in surrounding areas. My co-workers and I were fortunate in obtaining one of these pigeons, and from the brain we isolated a virus which we identified as the virus of the Eastern type of equine encephalomyelitis. This virus, as well as the strain obtained from human brain material, produced a fatal infection in normal pigeons.

This observation indicates that the domestic pigeon may be a host reservoir of the virus; indeed, it is possible that these birds may constitute a primary reservoir and that both horses and man are secondary hosts. If such is the case, the observation mentioned by Dr. Fothergill, that the disease in horses appeared almost simultaneously on both sides of Chesapeake Bay, is easily explained. In this regard it is interesting that one of the pigeon dealers noted that the "losses of pigeons stopped with the onset of cold weather"—an observation which bears out what has been repeatedly observed in epidemics of the disease in horses.

NEW YORK PATHOLOGICAL SOCIETY

ALFRED PLAUT, *President*

Nov. 18, 1938

ROBERT A. MOORE, *Secretary*

ADENOCARCINOMA OF THE JEJUNUM TREATED BY ENTEROENTEROSTOMY AND COMPLICATED BY INTUSSUSCEPTION. LESTER M. FRIEDLAND (by invitation) and M. F. WIENER.

A white man aged 68 was admitted to the Cumberland Hospital, Brooklyn, on Feb. 10, 1938, with a history of having lost 20 pounds (9.1 Kg.) in weight within the past four months and a complaint of abdominal pain, vomiting and constipation, increasing in severity and frequency, for the past ten days. The past history of the patient and the family history were irrelevant. The patient was cachectic; the temperature was 101.6 F.; the pulse, 106; the respirations, 26, and the blood pressure, 118 systolic and 64 diastolic. The heart and lungs were normal. The abdomen was soft and distended. There was visible peristalsis in the right half of the abdomen. No masses were felt.

The blood showed moderate polynucleosis and an increased urea nitrogen content (34 mg. in 100 cc.). The gastric analysis gave normal values. Roentgen examination showed distention of the intestines.

The patient was observed until February 11, when, under local anesthesia, an exploratory laparotomy was made through a midline incision. A hard annular constriction of the jejunum producing intestinal obstruction was discovered. A diagnosis of carcinoma of the jejunum was made. Because of the poor condition of the patient, resection was deferred, and a side to side anastomosis short-circuiting the constricted portion was performed.

There was marked improvement for forty-eight hours after the operation, followed by complicating parotitis and abdominal pain, distention and tenderness, and finally shock, from which the patient died on the twelfth postoperative day.

Autopsy.—The abdomen showed a clean laparotomy wound. The left inguinal region bulged and was found to contain gas communicating with that in the peritoneal cavity. The peritoneal cavity contained about 2,000 cc. of purulent fluid and foul-smelling gas. All surfaces of the intestine were covered with fibrin and were adherent. The heart weighed 460 Gm. The lungs showed bronchiectasia, pleural thickening, multiple calcified pleural nodules and bilateral apical fibrosis, with surrounding areas of caseation and calcification.

About 1.2 meters from the duodenojejunal junction was a large mass consisting of intussuscepted jejunum. When the outer layer was opened, the mucosa presented itself in the shape of a tube 30 cm. in length. The lumen of the distal end of this was markedly constricted by a fungating, partly necrotic and ulcerated annular adenocarcinomatous mass. Ten centimeters proximal to the mass a slit-like stoma of the recent anastomosis communicated with the internal cylinder of intestine. At one end of the stoma there was a perforation of the wall, 8 mm. in diameter, communicating with the peritoneal cavity.

The liver showed fat replacement and mild cirrhosis. The kidneys showed arteriolosclerotic changes and extratubular deposition of calcium. The prostate was hypertrophied.

Anatomic Diagnosis.—The following conditions were diagnosed: carcinoma of the jejunum; a jejunojejunostomy wound; intussusception of the jejunum; perforation of the jejunum (operative site); generalized peritonitis; benign hypertrophy of the prostate; hydronephrosis on the right; renal arteriolosclerosis and calcinosis; apical pulmonary tuberculosis (healed); nodular calcification of the

pleura; bronchiectasis; fibrous pleural adhesions; cardiac hypertrophy; replacement of the liver by fat; visceral congestion and cloudy swelling.

Carcinoma of the jejunum is rarely encountered, the largest series reported being that from the Mayo Clinic, which comprised 21 cases recorded up to 1929. A review of the literature to date fails to reveal any case similar to that described here.

The probable pathogenesis of the intussusception and the anatomic relations of the tumor and the enteroenterostomy are conceived to be as follows: The tumor produced obstruction, which was relieved by the short-circuiting enteroanastomosis. The latter was sufficiently near the neoplasm to be involved in the intussusception, which probably took place ante mortem.

Summary.—A thorough review of the literature reveals the infrequency of carcinoma of the jejunum. A case of this condition is reported here. The patient was treated by enteroanastomosis. This was followed by perforation, generalized peritonitis and preterminal intussusception.

SUPPURATIVE THROMBOPHLEBITIS OF THE AZYGOS VEIN ASSOCIATED WITH ABSCESS OF THE LUNG. JACOB FURTH.

Thrombophlebitis of the azygos vein is of importance from the standpoint of the pathogenesis of septic infections and their surgical control. There are only 2 cases of this disturbance on record, one reported by H. Nathan (*Virchows Arch. f. path. Anat.* **281**:430, 1931) and the other by E. Beer (*Ann. Surg.* **96**:687, 1932).

Our patient, a 57 year old man, acquired in 1936 an abscess of the lung, the etiologic background of which was not disclosed. Following resection of a rib and drainage of the abscess, he was well until five weeks after the operation; then pain appeared in the region of the fifth rib, and the temperature rose. Two months after the operation an abscess was noted in the wall of the chest and was drained. Ten weeks after the operation the patient died.

Postmortem examination showed fibroid pneumonia involving the greater part of the middle lobe of the right lung and adjacent parts of the upper and lower lobes, purulent bronchitis with slight bronchiectasis, and bronchopneumonia of all lobes. A small cavity, lined by thick fibrous connective tissue, marked the site of the drained abscess. Pleural adhesions bound the lungs to the chest wall. There were numerous abscesses in all lobes of the lung, most of them located peripherally. The posterior mediastinum was the site of suppurative inflammation with extensive fibrous adhesions and numerous pockets of pus. The azygos vein was embedded in this fibropurulent mass of tissue. Adherent thrombus filled the lumen of the distant third of the vein, and its remaining part was almost completely occluded by fibropurulent material. Friable red thrombi were seen in several small branches of the pulmonary artery, but the pulmonary veins appeared free from thrombi. The other organs were free from abscesses. A smear of the exudate of the azygos vein contained numerous mononuclear and polymorphonuclear leukocytes and many long chains of streptococci. The sputum contained hemolytic Staphylococcus aureus and nonhemolytic streptococci of the alpha type. The pulmonary abscess, pus from the azygos vein and blood from the right auricle of the heart, taken post mortem, yielded nonhemolytic alpha streptococci. Blood taken from veins of the arm during life and cultured was sterile.

The findings are in accord with the views of Schottmüller and Nathan on the pathogenesis of sepsis. In Nathan's case there was, in addition to thrombophlebitis of the azygos vein, thrombophlebitis of the pulmonary vein, and accordingly there were numerous abscesses in the greater circulation, including the kidneys and liver. In the present case only the pulmonary arteries were involved, and abscesses were not found in the organs supplied by the greater circulation.

IMMUNOLOGIC STUDIES WITH TRANSPLANTABLE AND VIRUS-INDUCED GROWTHS OF RABBITS. JOHN G. KIDD (by invitation).

An immunologic study of the Shope papilloma has shown that saline extracts of the growths contain a complement-binding antigen that is closely associated, if not identical, with the virus causing them (*J. Exper. Med.* **68**:703, 725 and 737, 1938). A similar study of the transplanted Brown-Pearce carcinoma has demonstrated that it, too, contains a specific complement-binding antigen, which is similar in its general traits to that derived from the virus-induced papillomas (*Proc. Soc. Exper. Biol. & Med.* **38**:292, 1938).

The antibody that reacts with the complement-binding antigen of the Brown-Pearce tumor has been found only in the serum of rabbits which bear the Brown-Pearce tumor or in which this tumor has retrogressed. It has not been present in the serum of the many normal rabbits tested, nor in the serum of rabbits with transplanted uterine cancers (Greene), of rabbits with virus-induced papillomas or of rabbits with various other diseases, including syphilis and vaccinia. The complement-binding antigen has regularly been found, and in considerable amount, in saline extracts of the Brown-Pearce tumor, wherever this is situated in the body. It has not been present, however, in extracts of the normal tissues of rabbits, in extracts of their uterine cancers or virus-induced papillomas or in extracts of rabbit tissues infected with vaccine virus or virus III.

Heating at 60 C. for thirty minutes has no great effect on the complement-binding antigen of the Brown-Pearce tumor, but heating at 65 C. inactivates it, as does also treatment with alcohol, treatment with acid to p_H levels of 2.5 and 4.5 and treatment with alkali to p_H levels of 10 and 11.5. In three experiments the antigen has passed readily through membranes with average pore diameters of 471, 397 and 383 microns and larger, but not through others with pore diameters of 348, 293 and 255 microns and smaller. The active material can be deposited readily with the high speed centrifuge, all of it coming down at 20,000 revolutions per minute for ninety minutes. The active material when purified by four differential centrifugations gives strongly positive results in the Millon, xanthoproteic and biuret tests for protein and a delayed Molisch reaction (immediately positive on hydrolysis). Spectroscopic examination of the purified active material from several sources has shown in each instance characteristic curves, with maximum absorption in the region of 2,600 angstroms (Lavin).

(The possible significance of the findings was discussed.)

DISCUSSION

JACOB FURTH: Dr. Kidd has convinced those present that both the papilloma and the Brown-Pearce carcinoma contain heavy substances, presumably proteins, with similar properties. I suppose, although he did not mention this, that one produces tumors and that the other is innocuous. I should like to hear about his attempts to produce tumors with the heavy protein of the Brown-Pearce tumor.

JOHN G. KIDD: So far as the experiments go, the antigenic material derived from the Brown-Pearce tumor has not proved pathogenic. The work has not gone far enough, however, to be conclusive.

JOEL WARREN: Is there any difference in the complement-fixing power of an extract of the papilloma of the domestic rabbit in contrast to the complement-fixing power of an extract of the growth in the cottontail?

JOHN G. KIDD: Extracts of growths from domestic rabbits, which contain little or no active virus, fix complement poorly or not at all. Extracts of papillomas from cottontails, containing much virus as a rule, fix it notably well.

JACOB FURTH: There is a minor question of importance to those who work in this field. The tables show that the titer of complement fixation was 1:320 with the crude extract, but only 1:40 with the virus concentrate obtained by high speed centrifugation, while the supernatant fluid was inactive. One would expect

the concentrated virus to be more active than the crude extract. How does Dr. Kidd explain this finding?

JOHN G. KIDD: The sedimented material used in the test was not concentrated; it was finally resuspended in the same volume of fluid as was present originally. I have a feeling that some of the virus and some of the complement-binding antigen remained in the supernatant fluid and stuck to the sides of the tubes in this experiment, as it undoubtedly has in other similar tests, and hence the amount of active material in the sediment was reduced in comparison with the crude material. Furthermore, if one throws material down hard in a centrifuge there is inevitably a good deal of aggregation of the sedimented stuff; not all of it can be resuspended in monodispersed form; much remains in clumps, and the clumped material is not very effective as a complement-fixing antigen. All these incidents, perhaps, account for the finding mentioned.

BIOPSY OF MATERIAL OBTAINED BY ASPIRATION IN THE DIAGNOSIS OF TUMORS. FRED W. STEWART (by invitation).

It is now eight years since diagnostic aspiration for the detection of neoplasms was first practiced in the Memorial Hospital for the Treatment of Cancer and Allied Diseases. Members of the staff claim no originality for their use of the method but only a rather extensive experience in the application of this very simple procedure. The exact number of cases in which a diagnosis of cancer was made by this method has not been determined, but in the past four years there have been upward of 2,600 cases in which a positive diagnosis of cancer has been rendered from material obtained through a 17 or 18 gage needle. The aspiration biopsy is never employed in cases of an accessible ulcerated tumor but only in cases in which either no tissue for biopsy could be obtained otherwise or in which circumstances make surgical excision of tissue for biopsy undesirable. The technic for obtaining material has already been described, and its exact fulfilment contributes notably to success in the use of the method. A negative report is of little significance and may be regarded as of value only when the clinical evidence is fully compatible with such a report. In the early days of the use of the method my associates and I viewed it with some hesitancy, a hesitancy fully shared by certain colleagues, who became vociferous in their objections. In our opinion, the method is becoming more useful year by year, and, properly interpreted, the smear is as valuable as the section. Naturally there are definite limits to interpretation of a smear, but one has to accept a method in the light of its usefulness rather than in that of its limitations.

One might refer to interpretation of a section as analytic pathologic examination. Interpretation of a smear, on the contrary, might be called synthetic pathologic examination, since one has to reconstruct the organized pathologic picture from loose cells and small aggregates of cells and rarely from one cell. To do so one must know all the clinical data in the case and especially the exact history of the patient and the status of the part subjected to aspiration of material. One cannot merely look at a smear and expect an immediate diagnosis of some specific type of cancer. If, however, one knows the age of the patient, the region involved and the history of the illness, and if one possesses knowledge of the types of tumors, benign and malignant, common to the region, one can from the isolated cells of a smear synthesize the pathologic picture and arrive at a proper diagnosis. (A demonstration of lantern slides followed.)

DISCUSSION

N. CHANDLER FOOT: I do not know why there has been so much "sales resistance" to the presentation of this method, and I think that after hearing Dr. Stewart's presentation tonight most of those present will see that there is nothing formidable about the method and that it ought to be employed much more than it is. It is being used at New York Hospital, with very good success, but

there has always been a disinclination on the part of the surgeons to use this method rather than biopsy of tissue obtained by excision. The only tumors with which my associates and I have had difficulty have been those of the stringy, fibrous, rather dry type sometimes encountered in the chest in the form of desmoid fibroma or of fibrosarcoma, which is well differentiated and very fibrous. Most of the other malignant tumors that we are interested in are more or less soft and apt to be granular, and there is not much difficulty in getting the material into the syringe. Dr. Stewart has shown the method. I think if one explains to the surgeon that if the vacuum is maintained, most, if not all, of the tumor cells that are detached will go into the syringe and not be transmitted elsewhere in the body, his fear of metastases will be allayed. In my opinion, there is not much ground for this fear. All the members have seen that most of these slides, even when shown as black and white lantern slides, are fairly readily diagnosed from their seats—one was so difficult that Dr. Stewart himself was not quite sure about it—but when a pathologist looks at these in the laboratory he knows where they come from, the surgeon has told him the source of the aspirated material and the history of the patient, and there are a great many other aids, among them that of color.

This leads me to the matter of stains. One can use a variety of stains; one is not limited to hematoxylin and eosin, and I think in studying material aspirated from lymph nodes one arrives at definite impressions, particularly in cases of Hodgkin's disease, if one uses a good Giemsa stain, for instance, and is fortunate in finding eosinophils in the aspirated material, which one usually does.

The interpretation of the evidence of tumor is sometimes difficult, but I believe that most of the difficulty is encountered in the early days of one's experimentation with the method. After one has experimented with it a little while, one acquires a good deal of insight and has no difficulty in making a satisfactory diagnosis. In the case of tumor of the breast in which an aspiration biopsy and later a radical operation have been done, it is always possible to have a biopsy of excised tissue at the time of operation, as a check up, and it is also possible to examine material obtained in the axillary dissection and see whether or not metastases have occurred there. So I see no objection to this method at all, and feel that it is not used as often as it should be.

A. R. CASILLI: My associates and I have a modest experience with the aspiration biopsy and our efforts have been more than compensated by the success we have had with it. A successful reading of the aspirated material depends on several factors. One of them is that the pathologist should have familiarized himself with the appearance of various tumor cells, and the best way to do that is to aspirate material from surgical specimens as a routine and study the cell pictures presented. A second factor is the eosin and hematoxylin stain, which is of fundamental importance. The third—and this offers an advantage over the ultimate paraffin section—is that with most aspiration biopsy slides one can reach a diagnosis within a short period, perhaps ten or fifteen minutes, after the time of aspiration.

AMOUR F. LIBER: What criteria are used in judging whether mucin is present? Are special stains necessary?

FRED W. STEWART: No.

AMOUR F. LIBER: How do you differentiate mucin and interstitial substances, such as those found in myxoid degeneration?

FRED W. STEWART: We are making diagnoses of cancer from smears; we are not interested in the specific nature of mucoids.

SOLOMON WEINTRAUB: My associates and I have not been fortunate in getting surgeons to use the aspiration biopsy, but internists have permitted us to make biopsies of this type on enlarged livers. Our method is somewhat different from that of Dr. Stewart. I wonder if he has used the method which we use. We

take a syringe containing physiologic solution of sodium chloride and equipped with a 13 gage needle. After we have struck the liver, we aspirate into the saline solution and remove the whole content with the needle. In that way we have been able to get enough material for diagnosis in every case. I wonder whether the saline method has been used at the Memorial Hospital.

FRED W. STEWART: We have not used it. We find the 18 gage needle perfectly satisfactory, and we want to keep the procedure as simple as possible. Just as soon as people began to write about the method, there appeared all kinds of complicated gadgets with which to get the tissue, and outfits were manufactured and sold for $25, whereas any tight needle and syringe can be used. We have always used the technic that I described, which can be carried out in any one's office.

Book Reviews

La réaction de fixation dans les tuberculoses humaines et animales. Achille Urbain. Second edition. Pp. 146. Price 28 francs. Paris: Masson & Cie, 1938.

The publication of this second edition of "Complement Fixation in Tuberculosis" was delayed by the necessity of completely revising the first edition. Although certain chapters were maintained within their primary limits, to others extensive additions were made, necessitated by the recent advances in this field. The volume is further augmented by a complete bibliography of the subject and an enlightening historical introduction. No detail, practical or theoretical, is omitted. If one wishes to read only about an interesting branch of scientific achievement, recorded from its inception at the turn of the century, this volume supplies both enlightenment and entertainment. The text is composed of seven chapters, beginning with the preparation of the elements of the reaction and their titration, and no details are lacking as to their preparation, the essential systems and finally (chapter 3) the technics of the reaction, in the presentation of which the methods of Calmette and Massol, Besredka and Petroff are elucidated. An additional chapter (4) is devoted to the tuberculous antibodies, their presence in organs and exudates, their transmission from mother to infant, the cutaneous reactions and tuberculous antibodies, the nature of these antibodies and their role. Chapter 5 is devoted entirely to complement fixation and the Vernes resorcinol seroflocculation and a comparison of the results of the two technics. The extensive studies in the field of diagnosis by complement fixation in tuberculosis become evident (chapter 6) only when the results in the various forms of localization of the disease (visceral, lymphatic, osteoarticular, cutaneous and seromembranous) are detailed. Finally, a chapter (7) is devoted to the application of complement fixation to the diagnosis of tuberculosis in animals. After completing the text, which is written in French, one cannot go far astray in concluding that this volume of 146 pages should find a place on the bookshelf of every serologist who can read French, as a ready reference to any phase of complement fixation in tuberculosis. It should be available also to every student of tuberculosis and medicine who faces the problem of being conversant with the various diagnostic phases of tuberculosis. Even though the application of fixation to the diagnosis of human tuberculosis is still disputed and the best figures admit only from 85 to 95 per cent positive reactions in cases of pulmonary tuberculosis, with 10 per cent positive reactions among nontuberculous persons, there are those who maintain that with proper reservations and discriminative evaluation the method is useful and of real interest in medicine when considered in the light of the results of other methods of examination. Among animals, the reaction is positive with from 90 to 98 per cent of those that have tuberculosis and negative with from 90 to 100 per cent of those that are not tuberculous. As a whole, this compact volume by Urbain is a good presentation of a difficult subject in a highly specialized field of medicine.

Surgical Pathology of the Diseases of the Neck. Arthur E. Hertzler, M.D., Surgeon to the Agnes Hertzler Memorial Hospital, Halstead, Kan., and Professor of Surgery in the University of Kansas. Pp. 237, with 206 illustrations. Price $5. Philadelphia: J. B. Lippincott Company, 1937.

This is the latest in a series of nine monographs on surgical pathology, with others to follow. The previous monographs dealt, in the order of their appearance, with diseases of bones, of the skin, of blood vessels, of muscles and nerves,

of the genitourinary tract, of the female generative organs, of the mammary glands, of the peritoneum, of the gastrointestinal tract and of the thyroid. Each dealt with an organ or a group of organs that functionally formed a natural unit.

The subject matter of the latest monograph is a region of the body with a variety of organs united only topographically. That deprives the book of some of the coherence which characterized the former monographs.

It has, as have the former monographs, the distinctive flavor of the author's personality in its style, in its humor and even in the treatment of the subject matter. This personality is the same that is responsible for the already apparent literary success of the recent autobiography of Hertzler.

The first chapter deals with a general review of surgical conditions of the neck; the following three chapters take up Hodgkin's granuloma, lymphosarcoma, lymphoepithelioma and endothelioma of the lymph nodes (which Hertzler persists in calling lymph glands). Rare primary tumors (of the carotid body, of the nerves and so forth) and tumors due to developmental abnormalities are discussed in the fifth and sixth chapters. The remaining four chapters have to do with benign tumors, with inflammations and tumors of the salivary glands, with metastatic growths and with inflammatory lesions of the neck.

The book will be of considerable interest to the surgeon and ought to provide pleasant reading for the pathologist, although the informed pathologist will find in it relatively little factual information.

The illustrations are abundant and excellent; the photographs of specimens and especially the photomicrographs would gain if the magnification were stated.

The bibliographic references are intended to be selective. The index fills 9 pages and is well organized. The paper, print and binding are excellent.

La tuberculose pulmonaire chez les sujets apparemment sains et la vaccination anti-tuberculeuse. L. Sayé, Professor de Phthisiologie à l'Université de Barcelone. Paper. Pp. 256, with 88 illustrations. Price, 60 francs. Paris: Masson & Cie, 1938.

This timely monograph on the occurrence of tuberculosis among apparently normal persons summarizes the numerous publications of recent years dealing with the epidemiology, pathology, diagnosis, therapeutics and prophylaxis of tuberculosis. There is definite evidence that the rate of tuberculization in urban communities is falling. However, roentgenologic demonstrable pulmonary lesions of tuberculosis have been found in from 1 to 3 per cent of persons apparently well. The incidence of tuberculosis among soldiers, students, nurses and other groups is summarized. Of the newer problems in the field of pathology, the occurrence and the mode of production of epituberculosis are discussed. Sayé concludes that epituberculosis results from compression of bronchi by enlarged nodes. This condition is at times associated with the presence of tubercle bacilli in the sputum. The relative importance of physical, fluoroscopic and roentgenographic examination, the importance of the tuberculin test and the demonstration of tubercle bacilli are discussed in the chapter dealing with the diagnosis of tuberculosis. Sayé considers fluoroscopic examination important but inferior in value to roentgenographic examination. In his discussion of allergy he mentions that the intracutaneous test has greater sensitivity than the Pirquet test. In his own investigations he has used Koch's old tuberculin in amounts up to 10 mg. With the purified protein derivative of the tubercle bacillus the reactions were less marked than with old tuberculin, but the percentage of positive reactors was the same with both tuberculins. The result obtained by microscopic examination of sputum and gastric contents for tubercle bacilli is inferior to that obtained by either cultural procedures or inoculation of animals. Sayé devotes considerable space to the use of the BCG vaccine. He is convinced of its innocuousness and its value in protecting against tuberculosis and believes that this vaccine will play an important role in the control of tuberculosis. In conclusion he discusses the

importance of periodic examination of contacts. The style is terse, and the numerous tables summarizing the results of various investigators are highly informative.

A Textbook of Bacteriology. Thurman B. Rice, A.M., M.D., Professor of Bacteriology and Public Health at the Indiana University School of Medicine. Second edition. Cloth. Pp. 563, with 121 illustrations. Price $5. Philadelphia: W. B. Saunders Company, 1938.

In the preface Rice expresses himself as feeling "that the book has been highly successful." The present revision retains the plan of the first edition, namely, that of providing a relatively simple textbook of such size that students may be expected to master it in one semester. The book is intended mainly for the medical student. It covers bacteriology and the applications of the science to practical medicine in considerable detail, and it may well be doubted whether students really can "master it in one semester." But all the topics of the 58 chapters are not of equal importance to the student, and there is room for condensation and even elimination if "the textbook should contain only those phases of the subject as are rather definitely established." By way of illustration reference may be made to the discussion of "antivirus" on page 475 and elsewhere. Here and there a tendency to dogmatism has resulted in pronouncements the soundness of which may be questioned. For example: Is there reliable evidence that the Semple vaccine is of value in preventing rabies in dogs? Is it established that "aggressins" and "antiaggressins" exist as specific substances? Is it accepted that human convalescent serum is of value in the treatment of infantile paralysis? Is it known that foreign proteins "after being precipitated, are phagocytized and in that way digested so that they can no longer do harm?" Is it sound practice to permit or arrange for children to have measles "when they can be given proper care"? Does any one know that there is increased susceptibility to infection in the "negative phase"? Is it definitely settled that tetanus toxin is not absorbed by any other cells than nerve cells and that it is not conveyed to the central nervous system by the blood? No mention is made of lymphocytic choriomeningitis, venereal lymphogranuloma and the Frei test or of the remarkable recent advances in the investigation of yellow fever. The omission of all bibliographic references robs the independent student of guidance to outside reading and tends to lower the scientific level of the presentation.

The Harvey Lectures Delivered under the Auspices of the Harvey Society of New York, 1937-1938. Under the patronage of the New York Academy of Medicine. Series XXXIII. Cloth. Pp. 275, with illustrations. Price $4. Baltimore: Williams & Wilkins Company, 1938.

This volume contains the following lectures: The Nature of the Visual Process, by Selig Hecht, professor of biophysics, Columbia University; the Pasteur-Meyerhof Reaction in Muscle Metabolism, by Einar Lundsgaard, professor of physiology, University of Copenhagen; The Functional Significance of the Lymphatic System, by C. K. Drinker, professor of physiology, Harvard University School of Public Health; Transfer of Water and Solutes in the Body, by John P. Peters, professor of medicine, Yale University; The Cortical Representation of Somatic Sensibility, by Philip Bard, professor of physiology, Johns Hopkins University; The Isolation and Properties of Tobacco Mosaic and Other Virus Proteins, by Wendell M. Stanley, the Rockefeller Institute for Medical Research, Princeton, N. J.; The Chemistry and Biology of Male Sex Hormones, by F. C. Koch, professor of biochemistry, University of Chicago; Experimental Hypertension Induced by Renal Ischemia, by Harry Goldblatt, professor of experimental pathology, Western Reserve University. These lectures review ably recent advances in the fields of research represented.

A Critical Investigation of the Blood Groups and Their Medico-Legal Application. Dawood Matta, M.B., Ch.B. (Cairo), Ph.D. (Glasgow), Lecturer on Forensic Medicine, Faculty of Medicine, University of Egypt, Cairo. Pp. 231. Cairo: The Egyptian University, 1937.

The main thesis of this study is the demonstration of the existence in groups B and AB of subgroups analogous to the subgroups of groups A and AB. On this basis human beings could be divided into the following blood types: O, A_1, A_2, B_1, B_2, A_1B_1, A_1B_2, A_2B_1 and A_2B_2. Matta has produced a number of anti-O reagents by suitably diluting and absorbing immune serums obtained by injecting human blood of group O into certain rabbits and goats. Such serums, according to Matta, act intensely on all O bloods, somewhat less strongly on bloods of subgroups A_2 and B_2, only moderately on blood of subgroup A_2B_2 and weakly or not at all on bloods of the other subgroups. The author asserts that the published exceptions to Bernstein's theory are probably valid. He therefore proposes a new hypothesis to explain the inheritance of the blood groups and to account for the existence of the subgroups. However, there are weaknesses in his arguments against Bernstein's theory and obvious flaws in his own theory of inheritance. The inheritance of the blood groups, subgroups and M-N types was studied in a series of 104 families with 315 children, and no exception was encountered to Bernstein's theory of the inheritance of the blood groups or to the theory of the inheritance of the M-N types held by Landsteiner and Levine. Matta also describes some of his experiences in determining blood groups on dried blood stains and secretions.

Human Pathology. Howard T. Karsner, M.D., Professor of Pathology, Western Reserve University, Cleveland, Ohio. With an Introduction by Simon Flexner, M.D. Fifth edition, revised. Cloth. Pp. 1013, with 461 illustrations. Price, $10. Philadelphia: J. B. Lippincott Company, 1938.

The first edition of this book was published in 1926. The purpose of the work was and is "to present the morphologic alterations incident to disease in the light of modern views as to their functional significance." The issue of a new and revised edition approximately every third year shows that the book has met a definite need. The present edition presents a thorough revision of every chapter, new illustrations and new references. No change has been made in the general arrangement of the book. Human pathology is presented in the two traditional divisions of general and special. The field usually covered by general pathology has been reduced somewhat, and wisely so, by limiting the chapter on infection to general principles. The author writes (page 219): "The phenomena of resistance and immunity are covered in special texts. The pathology of many of the special infections must be sought in the sections on special pathology or in books and original articles on the subject." The division of special pathology, as customary, includes all the organ systems except the skin, eye and ear. Karsner's book is well arranged and well written; it will be a reliable guide for the student and useful for reference.

A Textbook of Histology. Alexander A. Maximow, late Professor of Anatomy, University of Chicago, and William Bloom, Associate Professor of Anatomy, University of Chicago. Third edition. Cloth. Pp. 668, with 542 illustrations. Price $7. Philadelphia: W. B. Saunders Company, 1938.

This book needs no detailed introduction. It has achieved the position of a standard text, noted for the competence and clearness of its presentation and the excellence of its illustrations. The present edition has been revised thoroughly and includes the recent advances in histology and "histophysiology." In the explanation of the relation of function to structure, facts are taken from pathology as well as from physiology. Forty-two new illustrations have been added, including one in colors of the cells of the human marrow. It is a pleasure to add that the publisher has done his part most creditably.

Books Received

ANNUAL REPORT OF THE MEDICAL DEPARTMENT FOR YEAR ENDED 31ST DECEMBER, 1937, INCLUDING THE ANNUAL REPORT OF THE MEDICAL LABORATORY, DAR ES SALAAM, TANGANYIKA TERRITORY. Paper. Pp. 92. Price 4 shillings. Dar es Salaam: Government Printer, 1938.

REPORT ON RADIUM BEAM THERAPY RESEARCH 1934-1938. Constance A. P. Wood, L. G. Grimmet, T. A. Green and others. Medical Research Council Special Report Series, no. 231. Pp. 75. Price $1.20. London: His Majesty's Stationery Office, 1938.

HANDBUCH DER VIRUSFORSCHUNG. Herausgegeben von Prof. Dr. R. Doerr, Basel, and Prof. Dr. C. Hallauer, Berne. Erste Hälfte. Paper. Pp. 546, with 71 illustrations. Price 66 reichsmarks. Vienna: Julius Springer, 1938.

ÉTUDE MORPHOLOGIQUE ET BIOLOGIQUE SUR LES FLAGELLÉS INTESTINAUX PARASITES DES MURIDÉS. ÉTUDE COMPARATIVE DES FLAGELLÉS DU COBAYE. Lèon Morénas. Annales de l'Université de Lyon. 3e serie, Médecine. Fasc. 1. Paper. Pp. 234, with 12 illustrations. Price 60 francs. Paris: Masson & Cie, 1938.

SURGICAL PATHOLOGY OF THE DISEASES OF THE MOUTH AND JAWS. Arthur E. Hertzler, M.D., Surgeon to the Agnes Hertzler Memorial Hospital, Halstead, Kan., and Professor of Surgery, University of Kansas. Cloth. Pp. 248, with 206 illustrations. Price $5. Philadelphia: J. P. Lippincott Company, 1938.

ANIMAL PATHOLOGY. Russell A. Runnells, D.V.M., M.S., Associate Professor of Veterinary Pathology, Iowa State College. Cloth. Pp. 464, with 127 illustrations. Price $6. Ames, Iowa: Collegiate Press, Inc., 1938.

ANNUAL REPORT OF THE SURGEON GENERAL OF THE PUBLIC HEALTH SERVICE OF THE UNITED STATES FOR THE FISCAL YEAR 1938. Cloth. Pp. 184. Price 60 cents. Washington, D. C.: U. S. Government Printing Office, 1938.

THE PATIENT IS THE UNIT OF PRACTICE. Duane Willard Propst, A.B., B.S., M.D., Assistant Professor of Medicine, University of Illinois College of Medicine. Cloth. Pp. 219. Price $3.50. Springfield, Ill.: Charles C. Thomas, Publisher, 1939.

MEASLES. REPORT OF THE MEDICAL OFFICER OF HEALTH AND SCHOOL MEDICAL OFFICER ON THE MEASLES EPIDEMIC 1935-1936. Paper. Pp. 94. Price 1 shilling. London: London County Council, 1938.

ARBEITEN AUS DEM SERO-BAKTERIOLOGISCHEN INSTITUT DER UNIVERSITÄT HELSINKI. Herausgegeben von Prof. Dr. Osw. Streng. Vol. 10 (1937-1938). Various pagination. Helsinki, 1938.